THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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EDITOR'S NOTE

Through an error, the title of the abstract by A. A. Schaeffer on p. 374 of the October issue was incorrectly printed. The correct title should be: "Differences between Scottish and American amebas of the species *Chaos diffluens* Müller," not "*Chaos chaos* Müller."

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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FIFTIETH YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio*, who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen: Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1937.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1937, the book value of the Endowment Funds in the hands of the Central Hanover Bank and Trust Company as Trustee, was

General Fund,	Securities	$ 915,302.04
	Real Estate	9,595.71
	Cash	867.87
Library Fund,	Securities	174,141.23
	Real Estate	20,000.00
	Cash	102.85
		$1,120,009.70

The income collected from these Funds was as follows:

General Endowment	$39,945.92
Library Fund	7,345.93
	$47,291.85

Of this $4,899.39 represented the collection of arrears of interest outstanding at the beginning of 1937, reducing this item to

Arrears *General Fund*	$10,105.86
Arrears *Library Fund*	2,650.00
	$12,755.86

The dividends from the General Biological Supply House amounted to $10,160.

Retirement Fund. A total of $4,060 was paid in pensions. The Fund at the end of the year consisted of securities of the book value of $15,769.01

Real Estate	2,260.00	
Cash	212.82	$18,241.83

The interest in arrears has been reduced to less than $5.00.

Plant Assets. The land (exclusive of Gansett and Devil's Lane), buildings, equipment and library, represented an investment of $1,777,408.38

less reserve for depreciation	495,772.66	$1,281,635.72

Income and Expenses. Expenses including $24,343.77 depreciation exceeded income by $4,370.22.

There was expended from current funds net $19,549.01 for plant account.

After careful consideration by the Executive Committee and the accountants the rate of depreciation was changed as follows:

	Old Rate per annum	*New Rate per annum*
Buildings		
Brick	2%	1 %
Wood, etc.	2%	2 %
Equipment	5%	5 %
Library		
Back sets	5%	none
Other	5%	2½%

At the end of the year the Laboratory owed on mortgage $8,500, notes payable $10,500, and accounts payable $5,947.12. It had accounts and notes receivable of $13,562.36 and $12,298.54 in cash and bank accounts in its current assets.

Following is the balance sheet, the condensed statement of income and outgo, and the surplus account all as set out by the accountants:

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1937

Assets

Endowment Assets and Equities:		
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee—Schedules I-a and I-b	$1,120,009.70	
Securities and Cash—Minor Funds—Schedule II .	8,513.51	$1,128,523.21

Plant Assets:			
Land—Schedule IV	$ 110,884.58		
Buildings—Schedule IV	1,238,924.01		
Equipment—Schedule IV	162,833.58		
Library—Schedule IV	264,766.21	$1,777,408.38	
Less Reserve for Depreciation		495,772.66	
		$1,281,635.72	
Cash in Dormitory Building Fund		223.24	
Cash in Reserve Fund		24.65	$1,281,883.61
Current Assets:			
Cash		$ 12,298.54	
Accounts and Notes—Receivable		13,562.35	
Inventories:			
Supply Department	$ 38,282.75		
Biological Bulletin	12,764.61	51,047.36	
Investments:			
Devil's Lane Property	$ 43,916.11		
Gansett Property	5,718.49		
Stock in General Biological Supply House, Inc.	12,700.00		
Securities and Cash—Retirement Fund—Schedule V	18,241.83	80,576.43	
Prepaid Insurance		3,657.61	
Items in Suspense (Net)		71.65	$ 161,213.94
			$2,571,620.76

Liabilities

Endowment Funds:			
Endowment Funds—Schedule III	$1,119,594.24		
Reserve for Amortization of Bond Premiums	415.46	$1,120,009.70	
Minor Funds—Schedule III		8,513.51	$1,128.523.21
Plant Liabilities and Funds:			
Mortgage—Payable, Howes Property		$ 8,500.00	
Notes—Payable a/c Bar Neck Property Purchase		8,500.00	
Donations and Gifts—Schedule III		1,034,699.61	
Other Investments in Plant from Gifts and Current Funds		230,184.00	$1,281,883.61
Current Liabilities and Surplus:			
Accounts—Payable		$ 5,947.12	
Notes—Payable		2,000.00	
		$ 7,947.12	
Current Surplus—Exhibit C		153,266.82	$ 161,213.94
			$2,571,620.76

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1937

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund ...		$ 39,945.92		$ 39,945.92
Library Fund		7,345.93		7,345.93
Donations		36.68		36.68
Instruction	$ 8,219.29	10,005.00		1,785.71
Research	4,281.68	16,695.00		12,413.32
Evening Lectures	33.50		$ 33.50	
Biological Bulletin and Membership Dues	9,282.60	9,712.40		429.80
Supply Department—Schedule VI	43,869.93	39,640.88	4,229.05	
Mess—Schedule VII	28,051.56	28,647.58		596.02
Dormitories—Schedule VIII ..	25,813.00	13,264.18	12,548.82	
(Interest and Depreciation charged to above three Departments—See Schedules VI, VII, and VIII)	*23,698.07*			23,698.07
Dividends, General Biological Supply House, Inc.		10,160.00		10,160.00
Rents:				
Bar Neck Property		3,973.95		3,973.95
Bay Shore Property	249.55	132.00	117.55	
Howes Property	701.26	480.00	221.26	
Janitor's House	18.85	360.00		341.15
Newman Cottage	142.30	250.00		107.70
Danchakoff Cottage	282.79	750.00		467.21
Sale of Duplicate Library Sets		153.04		153.04
Interest on Notes—Receivable .		240.00		240.00
Sundries		70.76		70.76
Maintenance of Plant:				
Buildings and Grounds	23,733.29		23,733.29	
Chemical and Special Apparatus Expense	14,355.36		14,355.36	
Library Expense	7,639.80		7,639.80	
Truck Expense	1,023.44		1,023.44	
Workmen's Compensation Insurance	472.30		472.30	
Sundry Expense	46.17		46.17	
General Expenses:				
Administration Expense	14,713.71		14,713.71	
Endowment Fund Trustee and Safe-keeping	1,001.95		1,001.95	
Interest on Notes and Mortgage—Payable	639.11		639.11	
Bad Debts	1,016.40		1,016.40	
Reserve for Depreciation	24,343.77		24,343.77	
	$186,233.54	$181,863.32	$106,135.48	$101,765.26

Excess of Expenses over Income carried to Current Surplus—Exhibit C	4,370.22	4,370.22
	$186,233.54	$106,135.48

EXHIBIT C

Marine Biological Laboratory, Current Surplus Account, Year Ended December 31, 1937

Balance, January 1, 1937			$156,677.06
Add:			
Reserve for Depreciation charged to Plant Funds			24,343.77
			$181,020.83
Deduct:			
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,			
Land		$ 1,135.19	
Buildings		361.17	
Equipment		3,823.32	
Library		14,388.83	
		$19,708.51	
Less Received for Plant Assets Disposed of		159.50	
		$19,549.01	
Pensions and Allowances Paid	$4,060.00		
Expenses on account of Retirement Fund Securities	540.38		
	$4,600.38		
Less Retirement Fund Income	765.60	3,834.78	
Excess of Expenses over Income as shown in Exhibit B		4,370.22	27,754.01
Balance, December 31, 1937—Exhibit A			$153,266.82

Respectfully submitted,

LAWRASON RIGGS, JR.,
Treasurer

V. THE REPORT OF THE LIBRARIAN

A report of the expenditure of the budget assigned in 1937 at the usual total of $18,800 is as follows: books, $163.58; serials, $5,229.29; binding, $1,430.77; express, $103.65; supplies, $402.71; salaries, $7,150.00; back sets, $4,304.81; credit for sale of duplicates, $153.04; total, $18,631.77. The amount contributed by the Woods Hole Oceanographic Institution was separately accounted, $638.36.

Of the total number 1,330 current serials entered by the Library in 1937, the Marine Biological Laboratory purchased 378, the Woods Hole Oceanographic Institution 40; exchanged using the "Biological Bulletin" 621, using the Woods Hole Oceanographic Institution publications 75; received as gifts 216, seven of the last as gifts to the Woods Hole Oceanographic Institution. The number of books purchased was 47, eight of these out of the Woods Hole Oceanographic Institution budget. Gifts of 70 books came during the year, 34 from various publishers, 13 from the authors to the Marine Biological Laboratory, 21 from various sources, the greatest number having been presented by Prof. Frank R. Lillie, and 2 to the Woods Hole Oceanographic Institution direct from the author. By purchase 24 back sets of serials were completed by the Marine Biological Laboratory, 1 by the Woods Hole Oceanographic Institution; 12 partially completed by the former and 1 by the latter. By exchange of duplicate serials 8 further back sets were partially filled in for the Library.

The new reprints filed in 1937 totalled 7,042; current issues of 1936 but received this year 685 and these added to 1936 receipts total 1,940; 765 reprints current of 1937; reprints of earlier date, 4,337. Professor Frank R. Lillie presented 1,261 of these; Prof. E. W. Gudger, 100; and several hundred were presented by Prof. G. C. Wheeler; the remainder came to us from authors who filled in complete or nearly complete sets of their early reprints for us.

A summary of the above gives the interesting figures for the Library holdings as 1,330 current serial publications, 2,705 current (1936–37) reprints; 90 current books; 25 back sets of serials completed; 4,337 old reprints and 10 old books received; total bound volumes now in the Library, 43,442; and total reprints 102,022.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the fiftieth session of the Marine Biological Laboratory for the year 1937.

1. *Attendance.* Following an almost stationary registration of approximately 315 investigators during the depression years of 1932 to 1935 inclusive the attendance in 1936 showed a sharp increase, rising in that year to 359, which almost equalled the highest previous figure of 362 in 1931. During the year covered by this report a further increase has brought the number of investigators to 391, the largest in the entire history of the Laboratory. When to this number is added that of the students in the courses, which as for many years past has been about the

maximum that can be accommodated in the various class-rooms, the total attendance of 524 for 1937 is also seen to be the largest yet recorded, as is likewise the number of institutions represented (165 as compared with the next-highest figure of 158 in 1936).

While this rapid growth of the Laboratory, and in particular the widespread nature of its present scientific connections with American and foreign educational institutions, is most gratifying, it at the same time raises several serious problems. As has been pointed out in previous reports of the Director, neither the facilities of the Laboratory itself nor the living accommodations in the village of Woods Hole are such as to permit much further expansion; indeed, the registration for 1937 seems already to have passed the figure which experience has shown to be the optimum under existing conditions. Since there are several possible ways of meeting this situation, which must be faced without delay, and since the policies now adopted by the Laboratory are likely to affect profoundly the character of its future development, it has seemed wise to refer the question of attendance, as well as several others of importance, to the special Committee on Policy to which further reference will be made below.

The usual tabulation of the rise and fall of attendance by ten-day intervals for the past 10 years follows.

		1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937
May	30.....	7	15	9	6	6	8	11	12	11	14	10
June	10.....	50	64	55	50	51	54	46	54	43	41	57
"	20.....	114	140	139	153	153	127	129	137	127	142	145
"	30.....	212	240	197	208	217	172	184	196	174	190	214
July	10.....	247	281	238	253	258	225	235	249	226	242	285
"	20.....	247	282	242	250	273	245	253	256	232	260	301
"	30.....	245	272	249	253	281	248	255	248	257	272	320
August	10.....	234	250	256	254	302	257	261	264	245	282	311
"	20.....	208	226	243	245	280	236	244	250	235	266	307
"	30.....	168	183	220	204	239	190	205	211	192	223	248
September	10.....	110	112	157	122	136	129	117	93	94	121	126
"	20.....	50	43	59	44	69	58	45	38	26	47	39
"	30.....	12	14	14	8	14	13	12	9	11	20	14

2. *The Report of the Treasurer.* A comparison of the corresponding figures from the reports of the Auditors for 1936 and 1937 shows for the latter year a decrease in income from endowment of approximately $2,900, the actual amounts received for the two years in question having been $50,192.56 and $47,291.85. When account is taken of the fact that of the total sum received in 1937 $4,899.39 represented the collection of arrears rather than regular interest, it is obvious that the Laboratory at present is attempting care for the needs of the largest

number of investigators in its history with an income from endowment approximately $15,000 below its pre-depression level. That difficulties have been encountered in so doing is not surprising; but that these difficulties have so far been surmounted is due to the fine spirit of cooperation shown by all the investigators in adapting themselves to changed conditions. However, in view of the fact that the recent decline in the rates of interest obtainable from sound investments shows no indication of a reversal or even of a complete arrest, it is obvious that one of the most urgent needs of the Laboratory at present is for an increase in its endowment funds sufficient to bring its income from this source back at least to its pre-depression level.

Next in importance to its endowment as a source of income to the Laboratory are the subscriptions received from institutions and individuals for research space. The amount received from these sources in 1937 shows an encouraging increase of approximately $2,500 over the previous year and of more than $4,000 over the lowest level reached during the depression. It should be noted, however, that the sum received in 1937, while the highest in six years, still falls short by almost $1,500 of that received in 1931 when the attendance was appreciably smaller. In the early days of the Laboratory it was only through the support received from subscribing and coöperating institutions that its continued existence was possible. While in recent years the importance of this support may have seemed less obvious, it has in fact always been indispensable, and at no time more clearly so than at present.

Of the other usual sources of income, more favorable figures are shown by the Mess and the dormitories for 1937 than for several years past. This improvement is partly a real one due to an actual increase in income, resulting from the increased attendance and in particular from a lengthening of the most active season. In part, however, it is one of bookkeeping, due to the adoption of a new method for the estimation of depreciation which seemed to the Auditors and to the Executive Committee to be, on the whole, a fairer one than that previously used. This matter is discussed in considerable detail in a recent special report prepared by the Auditors, Messrs. Seamans, Stetson and Tuttle, which may be consulted for further information.

As for a number of years past, dividends received from the General Biological Supply House have been of material assistance in compensating for the decreases in sales by the Supply Department, which under the prevailing business conditions have been unavoidable.

3. *The Report of the Librarian.* During the year 1937 the growth of the library has continued at a rate which in view of the diminished income of the Laboratory from endowment funds may be considered to

be very satisfactory. While purchases of ordinary books and of back sets of journals were of necessity considerably curtailed in 1937, there was only a very slight decrease in the number of journals currently received. It is to the large number and convenient arrangement of these journals and their back sets that the library of the Marine Biological Laboratory owes its unique value to workers in Biology and related fields. Another extremely valuable feature of the library is its extensive collection of reprints which now exceeds 100,000, indexed and readily accessible to all workers at the Laboratory. The thanks of the Laboratory are due to those who in 1937, as well as in previous years, have generously contributed to this important collection. The following tabulation shows at a glance the character of the growth of the library during the past 10 years.

	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937
Serials received currently.....	874	985	1,060	1,080	1,126	1,137	1,197	1,271	1,339	1,330
Total number of bound volumes.	26,500	28,300	31,500	33,800	36,000	37,400	38,600	40,200	42,000	43,400
Reprints.....	51,000	59,000	64,000	70,000	76,000	81,000	86,000	92,000	95,000	102,000

4. *Changes in Personnel.* At the annual meeting of the Trustees held on August 10, 1937, the resignation as Director of Dr. M. H. Jacobs, who had become Associate Director of the Laboratory in 1925 and Director in 1926 and who now wished to be able to devote a larger proportion of his time to scientific research and writing, was received, and was accepted with expressions of regret. At the same time formal approval was given to the action of the President in having appointed several weeks previously a Special Committee to deal with the situation thus arising, of whose existence he and the Executive Committee had already been informed. Authority was given by the Board of Trustees to this Committee, which consisted of Drs. Calkins, Irving, Jacobs, Morgan, Parker, Stockard, and Conklin, Chairman, together with the Executive Committee, to take such steps as might be necessary to fill the vacant position. After several meetings of the Special Committee, in consultation with the Executive Committee, during the late summer of 1937 it had not proved feasible to arrive at a complete solution of the problem, but the important step had been taken of selecting for the position of Assistant Director, which had been vacant since 1925, Dr. Charles Packard of Columbia University, a man whose personal qualities and scientific attainments and long experience with the affairs of the Laboratory admirably fitted him to take over the administrative duties given up by the retiring Director on January 1, 1938.

5. *The Board of Trustees.* At the meeting of the Corporation held on Tuesday, August 11, Dr. T. H. Morgan, who as one of its most

distinguished members and as a Trustee since 1897 has contributed much to the present scientific standing of the Marine Biological Laboratory, was elected Trustee Emeritus. At the same meeting Dr. W. C. Curtis of the University of Missouri was chosen to fill the place thus created in the Class of 1941.

It is a painful duty to record the loss by death in very close succession in the spring of 1937 of three highly respected members or former members of the Board of Trustees: Dr. Milton Jay Greenman, who died on April 7, in his seventy-first year, Dr. William Morton Wheeler, who died on April 19, in his seventy-third year and Dr. Elias Potter Lyon who died on May 4, in his seventieth year. Suitable memorials commemorating the scientific achievements, and in particular the services to the Marine Biological Laboratory, of these three distinguished biologists will be presented at the next meeting of the Board of Trustees.

6. *Committee on Policy.* Several very important questions, of which that of the rapidly increasing attendance and the need for larger endowment funds mentioned above are typical, have for some time seemed to demand for their proper solution the collective wisdom and experience of a considerable number of persons who by close personal association with the affairs of the Marine Biological Laboratory have become thoroughly acquainted with its peculiar problems. Accordingly, the Board of Trustees at its meeting on August 11, 1937 authorized the President to appoint a Committee on Policy, not to exceed nine members, to consider the present status of the Laboratory and its policy for the future and to report at the next annual meeting of the Trustees. The committee appointed by the President, consisting of Drs. Calkins, Curtis, Goodrich, Jacobs, Morgan, Parker, Redfield, Stockard and Conklin, Chairman, has already held several very profitable meetings, with further discussion of various matters by correspondence, and its final report is now in the course of preparation.

7. *Gifts.* During the summer of 1937, Mr. Charles R. Crane, to whom the Laboratory is already so deeply indebted for material assistance and personal interest on numerous occasions in the past, added another much appreciated gift in the form of a very handsome sundial, in a setting appropriately beautified by plantings of grass and shrubs, opposite the main Brick Building by the side of Great Harbor. The sundial was designed by the late Professor Ernest Yalden, to whom it is a permanent and very fitting memorial. At the dedication exercises held in the Auditorium of the Laboratory on the morning of August 23 after introductory remarks by Dr. Frank R. Lillie the speech of presentation was made on behalf of Mr. Crane by Dr. H. C. Bradley.

and the speech of acceptance on behalf of the Laboratory by Dr. E. G. Conklin.

8. *The Cedar Swamp.* For many years an important collecting ground for certain types of biological material used by students in the courses as well as by investigators at the Laboratory has been the large Cedar Swamp lying to the southeast of the village of Woods Hole between the Falmouth road and the railroad. Swamps of this type are highly characteristic of the Cape Cod region but, in their primitive condition at least, are each year becoming more rare because of the cutting of timber, drainage, etc. When, therefore, during the past year an opportunity arose to purchase the tract in question from the New York, New Haven and Hartford Railroad, advantage was taken of it without delay, in order to preserve for the future use of Woods Hole biologists this very interesting region. The tract in question has an area of about 10 acres of which approximately half an acre is occupied by a shallow lake. Its greatest usefulness to the Laboratory both as a collecting ground and a suitable locality for future ecological studies will probably be secured by keeping it with a minimum of disturbance in its present wild condition.

9. *Lectures and Scientific Meetings.* During the summer of 1937 the number of regular evening lectures was 14, and in addition at 9 less formal evening meetings 37 shorter papers were presented and discussed. Of unusual interest was the final scientific meeting on August 26th and 27th, which was devoted exclusively to reports on work accomplished during the current season. The number of papers submitted for this meeting was so large that three full half-days were required for their presentation, with an additional half-day for demonstrations. Both in the number and excellence of its papers the 1937 meeting surpassed any previously held. Abstracts of nearly all the papers presented or read by title will be found in the October 1937 issue of the Biological Bulletin. Together they fill 38 pages of small print, and furnish very concrete evidence that the recent increases in attendance have not run ahead of the scientific productiveness of the Laboratory.

In addition to its own scientific meetings the Marine Biological Laboratory also acted as host to the Genetics Society of America, when between August 30th and September 1st it held its fourth and in many respects its most successful Woods Hole summer meeting.

There are appended as parts of the report:

1. The Staff, 1937.
2. Investigators and Students, 1937.
3. A Tabular View of Attendance, 1933–1937.
4. Subscribing and Coöperating Institutions, 1937.

5. Evening Lectures, 1937.
6. Shorter Scientific Papers, 1937.
7. General Scientific Meeting, 1937.
8. Members of the Corporation, August, 1937.

Respectfully submitted,

M. H. JACOBS,
Director.

1. THE STAFF, 1937

MERKEL H. JACOBS, *Director,* Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology Emeritus, The University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy Emeritus, Harvard University.
E. B. WILSON, Professor of Zoölogy Emeritus, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

T. H. BISSONNETTE, Professor of Biology, Trinity College.
P. S. CROWELL, JR., Instructor in Biology, Miami University.
C. E. HADLEY, Associate Professor of Biology, New Jersey State Teachers College at Montclair.
F. R. KILLE, Assistant Professor of Zoölogy, Swarthmore College.
S. A. MATTHEWS, Associate in Anatomy, School of Medicine, University of Pennsylvania.
L. P. SAYLES, Assistant Professor of Biology, College of the City of New York.
A. J. WATERMAN, Assistant Professor of Biology, Williams College

JUNIOR INSTRUCTORS

A. M. LUCAS, Associate Professor of Zoölogy, Iowa State College.
J. S. RANKIN, Teaching Fellow in Zoölogy, Amherst College.

PROTOZOÖLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
Elizabeth Drumtra Hughes, Instructor in Zoölogy, Wilson College.
G. W. Kidder, Instructor in Zoölogy, College of the City of New York.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

L. G. Barth, Assistant Professor of Zoölogy, Columbia University.
Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Viktor Hamburger, Assistant Professor of Zoölogy, Washington University.
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Oscar Schotté, Assistant Professor of Biology, Amherst College.

PHYSIOLOGY

I. Investigation

William R. Amberson, Professor of Physiology, University of Tennessee.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, The University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

Laurence Irving, Professor of Experimental Biology, University of Toronto.
Robert Chambers, Professor of Biology, New York University.
J. K. W. Ferguson, Assistant Professor of Physiology, Ohio State University.
Kenneth C. Fisher, Instructor in Zoölogy, University of Maine.
Rudolf Höber, Visiting Professor of Physiology, University of Pennsylvania.
C. Ladd Prosser, Assistant Professor of Physiology, Clark University.

Junior Instructors

F. J. M. Sichel, Instructor in Physiology, Howard University School of Medicine.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Michigan.
Francis Drouet, Research Fellow, Yale University.
G. W. Prescott, Assistant Professor of Biology, Albion College.

GENERAL OFFICE

F. M. MacNaught, Business Manager.
Polly L. Crowell, Assistant.
Edith Billings, Secretary.

RESEARCH SERVICE AND GENERAL MAINTENANCE

Samuel E. Pond, Technical Mgr.
O. W. Richards, Chemical Service.
G. Failla, X-ray Physicist.
T. E. Larkin, Superintendent.
W. C. Hemenway, Carpenter.
Lester F. Boss, Technician.
J. D. Graham, Glassblower.
J. T. Simonton, Assistant.
Elbert P. Little, X-ray.
R. T. Goffin, Technical Ass't.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Doris Endrejat, Mary A. Rohan, Assistants.

SUPPLY DEPARTMENT

James McInnis, Manager.
Milton B. Gray, Collector.
A. M. Hilton, Collector.
A. W. Leathers, Shipping Dept.
Geoffrey Lehy, Collector.
Walter Kahler, Collector.
Ruth S. Crowell, Secretary.
Anna N. Hall, Secretary.

THE GEORGE M. GRAY MUSEUM

George M. Gray, Curator Emeritus.

2. INVESTIGATORS AND STUDENTS, 1937

Independent Investigators

ABELL, RICHARD G., Fellow in Anatomy, University of Pennsylvania, School of Medicine.
ABRAMOWITZ, A. A., Research Assistant, Harvard University.
ADDISON, WILLIAM H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
ALLEE, W. C., Professor of Zoölogy, The University of Chicago.
AMBERSON, WILLIAM R., Professor of Physiology, University of Tennessee, School of Medicine.
ANDERSON, R. L., Professor of Biology, Johnson C. Smith University.
ANDREW, WARREN, Teaching Fellow, University of Georgia, School of Medicine.
APPEL, FREDERICK W., Associate Professor of Biology, St. John's College.
ARMSTRONG, PHILIP B., Professor of Anatomy, University of Alabama Medical School.
BAILEY, PERCY L., JR., Assistant Professor of Biology, College of the City of New York.
BALLARD, W. W., Assistant Professor of Anatomy and Biology, Dartmouth College.
BARGETON, DANIEL E., Assistant in Physiology at Faculty of Medicine, University of Paris.
BARTH, L. G., Assistant Professor of Zoölogy, Columbia University.
BEADLE, G. W., Assistant Professor, Harvard University.
BEAR, RICHARD S., Research Assistant, Washington University.
BENOIT, JACQUES, Department of Histology, Medical School, Strasbourg, France.
BISSONNETTE, T. HUME, Professor of Biology, Trinity College.
BOGDANOVITCH, SINISHA B., Assistant in the Department of Pharmacology, School of Medicine, Belgrade, Yugoslavia.
BOZLER, EMIL, Assistant Professor of Physiology, Ohio State University.
BRACHET, JEAN, Rockefeller Fellow, Princeton University.
BRADLEY, H. C., Professor of Physiological Chemistry, University of Wisconsin.
BRISBIN, G. W. F., University of Manitoba.
BRONFENBRENNER, J., Professor of Bacteriology, Washington University School of Medicine.
BROWN, DUGALD E. S., Assistant Professor of Physiology, New York University Medical College.
BUCK, JOHN B., National Research Fellow, California Institute of Technology.
CABLE, RAYMOND M., Assistant Professor of Parasitology, Purdue University.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CAMERON, JOHN A., Instructor, University of Missouri.
CANNAN, R. KEITH, Professor of Chemistry, New York University Medical College.
CAROTHERS, E. ELEANOR, Research Associate, University of Iowa.
CATCHESIDE, DAVID E., Lecturer, Botany School, Cambridge University, London, England.
CHAMBERS, ROBERT, Research Professor of Biology, Washington Square College, New York University.
CHENEY, RALPH H., Chairman and Professor of Biology, Long Island University.
CHIDESTER, FLOYD E., Professor of Zoölogy, Extension Division, College of Forestry, Syracuse University.
CHILD, GEORGE P., Instructor in Biology, Amherst College.
CLARK, ELEANOR LINTON, University of Pennsylvania, School of Medicine.
CLARK, ELIOT R., Professor of Anatomy and Director, Department of Anatomy, University of Pennsylvania, School of Medicine.

CLOWES, GEORGE H. A., Director of Research, Lilly Research Laboratories.
COHEN, ARTHUR, Seessel Research Fellow, Yale University.
COKER, R. E., Professor of Zoölogy, University of North Carolina.
COLE, KENNETH S., Assistant Professor of Physiology, College of Physicians and Surgeons, Columbia University.
CONKLIN, EDWIN G., Professor Emeritus of Biology, Princeton University.
COONFIELD, BENJAMIN R., Assistant Professor, Brooklyn College.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COREY, H. IRENE, Research Associate, University of Pennsylvania.
CO TUI, FRANK W., Associate Professor of Experimental Surgery, New York University Medical College.
COWDRY, E. V., Professor of Cytology, Washington University.
COX, EDWARD H., Professor of Chemistry, Swarthmore College.
CROASDALE, HANNAH T., Research Assistant in Physiology, Dartmouth College.
CROWELL, SEARS, Instructor in Zoölogy, Miami University.
CURWEN, ALICE O., Assistant Professor of Anatomy, Woman's Medical College of Pennsylvania.
DAHL, A. ORVILLE, Teaching Assistant, University of Minnesota.
DAVID, PAUL R., Columbia University.
DAVSON, HUGH, Rockefeller Fellow, University of London, London, England.
DENNY, MARTHA, Instructor in Zoölogy, Connecticut College.
DILLER, WILLIAM F., Assistant Professor, Dartmouth College.
DONALDSON, HENRY H., Member, Wistar Institute.
DORRIS, FRANCES, Research Asssitant in Embryology, Yale University.
DREYER, WILLIAM A., Instructor in Zoölogy, University of Cincinnati.
DROUET, FRANCIS, Research Fellow, Yale University.
DUBOIS, EUGENE T., Professor of Medicine, Cornell University Medical College.
DUGGAR, B. M., Professor of Physiological and Economic Botany, University of Wisconsin.
FAILLA, G., Physicist, Memorial Hospital, New York City.
FENN, W. O., Professor of Physiology, University of Rochester, School of Medicine and Dentistry.
FERGUSON, J. K. W., Assistant Professor of Physiology, Ohio State University.
FISCHER, ERNST, Associate Professor of Physiology and Pharmacology, Medical College of Virginia.
FISHER, KENNETH C., Instructor in Zoölogy, University of Maine.
FRISCH, JOHN A., Professor of Biology, Canisius College.
FRY, HENRY J., Visiting Investigator, Cornell University Medical College.
GARREY, W. E., Professor of Physiology, Vanderbilt University School of Medicine.
GAYLOR, JOHN B., Rockefeller Travelling Fellow, Rockefeller Foundation.
GOODRICH, H. B., Professor of Biology, Wesleyan University.
GOTTSCHALL, GERTRUDE, Assistant, Cornell University Medical College.
GRANT, RONALD, Assistant Lecturer in Zoölogy, University of Leeds, England.
GRAVE, CASWELL, Professor of Zoölogy, Washington University.
GREGERSEN, MAGNUS I., Professor of Physiology, University of Maryland, School of Medicine.
HADLEY, CHARLES E., Associate Professor of Biology, Montclair State Teachers' College.
HADORN, ERNST, Rockefeller Foundation Fellow, Zoölogical Institute, Berne, Switzerland.
HAMBURGER, VIKTOR, Assistant Professor, Washington University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HARVEY, ETHEL BROWNE, Princeton University.
HAYWOOD, CHARLOTTE, Associate Professor of Physiology, Mount Holyoke College.

HEILBRUNN, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
HENDEE, ESTHER C., Instructor in Biology, Russell Sage College.
HENSHAW, PAUL S., Biophysicist, Memorial Hospital, New York City.
HESS, WALTER N., Professor of Biology, Hamilton College.
HIBBARD, HOPE, Professor, Oberlin College.
HIESTAND, WILLIAM A., Assistant Professor of Physiology, Purdue University.
HILL, SAMUEL E., Assistant in General Physiology, Rockefeller Institute.
HÖBER, RUDOLF, Visiting Professor of Physiology, University of Pennsylvania.
HODGE, CHARLES, 4th, Instructor, Temple University.
HOFFMAN, OLIVE, Instructor in Medicine, University of Pennsylvania.
HOLLAENDER, ALEXANDER, Special Investigator, University of Wisconsin.
HOLMES, JOSEPH H., Department of Physiology, University of Maryland, School of Medicine.
HOPKINS, DWIGHT L., Assistant Professor, Duke University.
HOWE, H. E., Editor, Industrial and Engineering Chemistry.
HOWELL, CHARLES DEWITT, Graduate Student, Johns Hopkins University.
HUGHES, ELIZBETH DRUMTRA, Instructor in Biology, Wilson College.
HUGHES, ROSCOE D., Teaching Assistant, Columbia University.
HUNNINEN, ARNE V., Head of Biology Department, Oklahoma City University.
HUNTER, GEORGE W., III, Assistant Professor of Biology, Wesleyan University.
HUTCHINGS, LOIS M., Teacher of Biology, Weequahic High School, Newark, N. J.
IRVING, LAURENCE, Professor of Biology, Swarthmore College.
JACOBS, MERKEL H., Professor of General Physiology, University of Pennsylvania.
JENKINS, GEORGE B., Professor of Anatomy, George Washington University.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt University School of Medicine.
JOHNSON, FRANK H., Eli Lilly and Company Research Fellow, Princeton University.
JONES, E. RUFFIN, JR., Associate Professor of Biology, College of William and Mary.
JONES, RUTH MCCLUNG, Instructor in Botany and Zoölogy, Swarthmore College.
KEIL, ELSA M., Assistant Professor of Zoölogy, New Jersey College for Women.
KELTCH, ANNA K., Research Chemist, Eli Lilly and Company.
KEYES, DONALD B., Professor of Chemical Engineering, University of Illinois.
KIDDER, GEORGE W., Instructor in Biology, College of the City of New York.
KILLE, FRANK R., Assistant Professor of Zoölogy, Swarthmore College.
KINDRED, JAMES E., Associate Professor of Histology and Embryology, University of Virginia.
KNOWER, HENRY MCE., Research Associate in Biology, Yale University.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University, College of Medicine.
KOPAC, M. J., Research Associate in Biology, Washington Square College, New York University.
KORR, IRVIN M., Instructor, New York University Medical College.
KRAHL, MAURICE E., Research Chemist, Eli Lilly and Company.
KREEZER, GEORGE, Research Associate, The Training School, Vineland, New Jersey.
KRIEG, WENDELL J. S., Instructor in Anatomy, New York University, Medical College.
LANCEFIELD, D. E., Associate Professor of Zoölogy, Columbia University.
LANCEFIELD, REBECCA C., Associate, Rockefeller Institute.
LEVINE, DARWIN S., Teacher, Theodore Roosevelt High School, New York.
LILLIE, FRANK R., Professor of Embryology, Emeritus, The University of Chicago.
LILLIE, RALPH S., Professor of General Physiology, The University of Chicago.

Loeb, Leo, Professor Emeritus of Pathology, Washington University, School of Medicine.
Loeffler, Louis, Professor of Pathology, Middlesex College.
Lucas, Alfred M., Associate Professor, Iowa State College.
Lucas, Miriam Scott, Iowa State College.
Luyet, Basile J., Associate Professor of Biology, St. Louis University.
Lynn, W. Gardner, Instructor in Zoölogy, Johns Hopkins University.
McClung, Clarence E., Director, Zoölogical Laboratory, University of Pennsylvania.
Macdougald, Thomas J., Rockefeller Foundation Fellow in Experimental Cytology, Trinity College, Dublin, Ireland.
Maes, Julien, C. R. B., Fellow, Harvard University Medical School.
Magruder, S. R., Assistant in Anatomy, Cornell University Medical College.
Marsland, Douglas, Assistant Professor of Biology, Washington Square College, New York University.
Martin, W. E., Instructor in Biology, Purdue University.
Mathews, Albert P., Professor of Biochemistry, University of Cincinnati.
Matthews, Samuel A., Associate in Anatomy, University of Pennsylvania.
Mazia, Daniel, National Research Fellow, Princeton University.
Menkin, Valy, Instructor in Pathology, Harvard University Medical School.
Michaelis, Leonor, Member, Rockefeller Institute.
Miller, Faith Stone, Ohio University.
Miller, James A., Instructor in Zoölogy, Ohio University.
Mitchell, Philip H., Professor of Physiology, Brown University.
Morgan, Lilian V., Pasadena, California.
Morgan, T. H., Professor of Experimental Zoölogy, California Institute of Technology.
Morrill, Charles V., Associate Professor of Anatomy, Cornell University Medical College.
Morris, Samuel, Research Zoölogist, Temple University.
Murlin, John R., Professor of Physiology, University of Rochester.
Nebel, Bernard R., Associate in Research, Agricultural Experiment Station, Geneva, New York.
Netting, M. Graham, Curator of Herpetology and Lecturer in Biology, Carnegie Museum and University of Pittsburgh.
Newton, W. H., Reader in Physiology, University of London, England.
Nonidez, José F., Assistant Professor of Anatomy, Cornell University Medical College.
Northrop, John H., Rockefeller Institute for Medical Research.
Olson, Magnus, Research Assistant, University of Minnesota.
Orr, Paul R., Instructor, Brooklyn College.
Osterhout, W. J. V., Member, Rockefeller Institute for Medical Research, New York City.
Packard, Charles, Assistant Professor of Zoölogy, Columbia University.
Painter, Elizabeth S., Instructor in Physiology, University of Maryland, School of Medicine.
Parker, G. H., Professor of Zoölogy Emeritus, Harvard University.
Parmenter, Charles L., Associate Professor of Zoölogy, University of Pennsylvania.
Parpart, Arthur K., Assistant Professor of Physiology, Princeton University.
Patrick, Ruth, Professor of Botany, Pennsylvania School of Horticulture.
Pierce, Madelene E., Instructor, Vassar College.
Plough, Harold H., Professor of Biology, Amherst College.
Pollister, Arthur W., Assistant Professor of Zoölogy, Columbia University.
Potter, Truman S., Research Associate, The University of Chicago.
Poulson, Donald F., Instructor in Zoölogy, Yale University.
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SCHARRER, ERNST, Research Fellow, Neurologisches Institute, Frankfurt, Germany.
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SCHMITT, OTTO H., Washington University.
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SCHRAM, MILDRED W. S., International Cancer Research Foundation.
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TRAGER, WILLIAM, Assistant, Rockefeller Institute.
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YOUNG, DIXIE, Yale University.
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BAUER, KARL, University of Berlin.
BLACK, EDGAR C., Demonstrator in Experimental Biology, University of Toronto.
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CORSON, SAMUEL, Research Assistant in Cell Physiology, Washington Square College, New York University.
CULBRETH, SARAH E., Graduate Fellow, Duke University.
DERRICKSON, MARY B., Graduate Assistant, Duke University.
DETHIER, VINCENT G., Graduate Student, Harvard University.
DIENES, PRISCILLA A., Student, Radcliffe College.
DONNELLON, JAMES A., Student, University of Pennsylvania.
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HIATT, EDWIN P., Graduate Assistant, Duke University.
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ROSENFIELD, RICHARD, Medical Student, University of Pittsburgh.
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MORRIS, MARION C., Instructor, Washington University, School of Medicine.
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RAVITZ, LOUIS A., University of Wisconsin.
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PROTOZOÖLOGY

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INVERTEBRATE ZOÖLOGY

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BAIRD, ISABELLE, Graduate Assistant, Mount Holyoke College.
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GRIFFIN, DONALD R., Harvard University.
HAMILTON, HOWARD L., State University of Iowa.
HARROLD, CHARLES M., Wabash College.
HASKIN, HAROLD H., Assistant, Harvard University.
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STEELE, DEWEY G., Assistant Professor of Genitics, Connecticut State College.
STRANDINE, ELDON J., Northwestern University.
SUGARBAKER, JOHN D., Student, New Jersey State Teachers College.
SWANSON, CARL P., Student, Massachusetts State College.
WALKER, SALLY, Radcliffe College.
WARD, ELIZABETH B., Graduate Assistant in Zoölogy, University of Cincinnati.
WILLIAMS, MYRA A., Graduate Assistant in Zoölogy, Duke University.
WILSON, JAMES G., Graduate Assistant, University of Richmond.

3. TABULAR VIEW OF ATTENDANCE

	1933	1934	1935	1936	1937
INVESTIGATORS—Total	319	323	315	359	391
Independent	210	222	208	226	256
Under Instruction	66	49	56	76	74
Research Assistants	43	52	51	57	61
STUDENTS—Total	118	131	130	138	133
Zoölogy	54	54	55	55	57
Protozoölogy	11	11	16	17	16
Embryology	28	30	33	34	35
Physiology	19	23	20	22	16
Botany	6	13	6	10	9
TOTAL ATTENDANCE	437	454	445	497	524
Less Persons registered as both students and investigators	12	15	16	24	13
	425	439	429	473	511
INSTITUTIONS REPRESENTED—Total	120	131	143	158	165
By Investigators	92	98	111	120	134
By Students	58	75	70	77	79
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	1	1	–	2	3
By Students	2	5	3	3	2
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	5	4	7	9	16
By Students	–	1	1	5	–

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS IN 1937

American University
Amherst College
Atlanta University
Bowdoin College
Brown University
Bryn Mawr College
California Institute of Technology
College of Charleston
College of Physicians and Surgeons
Columbia University
Cornell University
Cornell University Medical College
C. R. B. Educational Foundation, Inc.
Dartmouth College
DePauw University
Duke University
General Education Board
Goucher College
Hamilton College
Harvard University
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Iowa State College
Johns Hopkins University
Kenyon College
Eli Lilly & Co.
Long Island University
Massachusetts State College
Medical College of Virginia
Memorial Hospital, New York City
Morehouse College
Mount Holyoke College
New York State Department of Health
New York University
New York University Medical School
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Purdue University
Radcliffe College
Rice Institute
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
St. John's College
Seton Hill College
Smith College
State University of Iowa
Swarthmore College
Syracuse University
Temple University
Union College
University of Chicago
University of Cincinnati
University of Georgia, School of Medicine
University of Illinois
University of Maryland Medical School
University of Minnesota
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Rochester Medical School
University of Virginia
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Wabash College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Wheaton College
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

5. EVENING LECTURES, 1937

Tuesday, June 29
Dr. W. T. Astbury ..... "The X-ray Interpretation of Protein Structure."

Friday, July 2
Dr. H. B. Goodrich ..... "The Development of Inherited Color Patterns in Fish."

Friday, July 9
Dr. E. Newton Harvey ..... "Electrical Potentials from the Human Brain."

Friday, July 16
Dr. Alexander Hollaender ..... "Studies in the Problem of Mitogenetic Radiation."

Wednesday, July 21
Dr. Edgar Allen ..... "Ovulation in Mammals and Internal Secretions Involved."

Friday, July 23
Dr. Leonor Michaelis ..... "The Problem of the Oxidation of Organic Compounds and Its Catalysis under Biological Conditions."

Friday, July 30
Dr. Caswell Grave ..... "Metamorphosis in Ascidians."

Thursday, August 5
Dr. F. Gudernatsch ..... "The Thymus Problem: Its Present Status after 25 Years of Experimentation."

Friday, August 6
Dr. Ernst Fischer ..... "Some Physical Aspects of Muscular Contraction."

Friday, August 13
Dr. John H. Northrop ..... "Concentration and Purification of Bacteriophage."

Wednesday, August 18
Dr. Robert Cushman Murphy .. "The Gates of the Antarctic."

Thursday, August 19
Mr. John S. Garth ..... "To Galapagos aboard Velero III"

Friday, August 20
Dr. H. C. Stetson ..... "The Submarine Canyons of the Continental Shelf."

Monday, August 30 (Under the joint auspices of the Genetics Society of America and the Marine Biological Laboratory)
Dr. Boris Ephrussi ..... "Aspects of the Physiology of Gene Action."

6. SHORTER SCIENTIFIC PAPERS, 1937

Tuesday, July 6

Dr. Ethel Browne Harvey ..... "Parthenogenetic Merogony in the Naples Sea-urchins."

Dr. Jean Brachet ..... "Some Oxidative Properties of Isolated Amphibian Germinal Vesicles."

Dr. Albert Tyler ..... "Influence of Temperature and Other Agents on the Respiration and Development of Marine Eggs."

Tuesday, July 13

Dr. Warren Andrew ..... "Effects of Fatigue Due to Muscular Exercise on the Purkinje Cells of the Cerebellum of Mice at Various Ages."

Mr. Zareh Hadidian,
Mr. Milton S. Dunn and
Dr. Roland Walker ..... "Localization in the Oculomotor Nuclei of the Goldfish."

Dr. Ernst Scharrer ..... "Some New Observations on the Secretory Activity of Neurons."

Dr. C. Ladd Prosser ..... "Synaptic Transmission in the Sixth Abdominal Ganglion of the Crayfish."

Tuesday, July 20

Dr. L. G. Barth ..... "Chemical Stimulation of the Amphibian Ectoderm."

Dr. Viktor Hamburger ..... "Limb Bud Transplantation in Chick Embryos."

Dr. Oscar E. Schotté ..... "Adult Organizers and Their Action in Adult Tissues."

Dr. L. S. Stone ..... "The Development of a Salamander, Amblystoma punctatum," shown by motion pictures.

Tuesday, July 27

Dr. J. K. W. Ferguson and
Dr. F. A. Hitchcock ..... "The Effect of Standing on the Carbon Dioxide Content of Alveolar Air and Total Ventilation Volume."

Dr. Eugene F. Dubois and
Dr. James D. Hardy ..... "The Mechanism of the Loss of Heat from the Human Body."

Dr. Emil Bozler ..... "Peripheral Inhibition of Smooth Muscle."

Dr. William R. Amberson,
Dr. Thomas P. Nash,
Dr. Arthur G. Mulder and
Miss Dorothy Binns ..... "The Relationship of Tissue Chloride to Blood Chloride."

Monday, August 2

Dr. Ruth Patrick ..... "The Use of Diatoms from Geological Excavations at Clovis, New Mexico, as Indicators of Water Conditions."

Mr. H. N. Stoudt ..... "Gemmipary in Kalanchoe rotundifolia and Other Crassulaceae."

Mr. A. O. Dahl ..... "Pollen Analysis of the Air in Relation to Hay-fever."

Tuesday, August 3

Dr. P. W. Whiting ..... "A Convenient Test of Physical Agents as Producers of Dominant Lethals."

Dr. Bernard R. Nebel ..... "Cytological Observations on Colchicine."

Dr. R. Weissenberg ..... "Demonstration of Vital Staining Preserved in Paraffin Sections of Lamprey Embryos." (Bismark Brown Method.)

Dr. William R. Duryee ..... "Microfilm on Some Experiments on Isolated Amphibian Germinal Vesicles."

Dr. Robert Chambers ..... "Cortical Cytolysis of the Echinoderm Egg."

Tuesday, August 10

Dr. Francis M. Summers ..... "Some Aspects of Normal and Regulative Development in the Colonial Ciliate, Zoöthamnium alternans."

Dr. A. A. Schaeffer ..... "Morphology, Behavior and Reproduction in Type A and Type B of Chaos chaos Linnaeus, the Giant Multi-nucleate Ameoba of Roesel."

Dr. Theodor von Brand ..... "Observations upon the Chemical Composition and the Metabolism of a Larval Parasitic Nematode."

Dr. Paul S. Galtsoff ..... "Observations and Experiments on Sex Change in the Adult American Oyster, Ostrea virginica."

Tuesday, August 17

Dr. Albert Tyler and
Mr. Norman H. Horowitz ..... "A Sea Water Buffer for Marine Eggs."

Mr. Edgar C. Black and
Dr. Laurence Irving ..... "The Effect of CO_2 upon the Oxygen Capacity of the Blood of Some Fresh-Water Fish."

Dr. Irvin M. Korr ..... "Oxidative Mechanisms in the Resting and Fertilized Sea-urchin Egg."

Dr. Kurt G. Stern and
Mr. Delafield Dubois ..... "Methods for the Study of Rapid Chemical Reactions and Their Application to the Kinetics of Enzyme-Substrate and Enzyme-Inhibitor Compound Formation."

Tuesday, August 24

Dr. B. Luyet ..... "Mechanism of Cellular Death by Freezing."

Dr. D. Mazia ..... "Binding and Penetration of Bivalent Cations in Elodea Cells."

Dr. G. H. A. Clowes,
Miss A. K. Keltch and
Dr. M. E. Krahl ..... "Factors Governing Cellular Responses to Nitro and Halo Phenols."

Dr. M. E. Krahl,
Dr. G. H. A. Clowes and
Miss A. K. Keltch ..... "The Possible Rôle of Acidic Dissociation in the Physiological Effects Produced by Nitro and Halo Phenols."

Dr. Rudolf Höber and
Dr. Bernard R. Nebel ..... "Depolarization of Muscle and Nerve Membranes by Organic Substances."

Dr. A. E. Navez and
Dr. Ethel Browne Harvey ..... "Indophenol Oxidase in the Arbacia Egg and the Nadi Reagent."

Thursday, September 2

Dr. Theodore Shedlovsky ..... "The Ionization of Carbonic Acid."

7. GENERAL SCIENTIFIC MEETING, 1937

Thursday, August 26

Dr. B. J. Luyet and
Mr. E. L. Hodapp ..... "On Some Conditions Determining Sub-cooling in Plant Tissues."

Dr. B. J. Luyet and
Sister P. M. Gehenio ..... "On the Double Freezing Point of Some Living Tissues."

Dr. H. J. Curtis and
Dr. K. S. Cole ..... "Transverse Electric Impedance of the Squid Giant Axon."

Dr. K. S. Cole and
Mr. J. M. Spencer ..... "Electric Impedance of Suspensions of Unfertilized and Fertilized Arbacia Eggs."

Dr. K. S. Cole and
Dr. H. J. Curtis ..... "Electric Impedance of Single Arbacia Eggs."
Dr. Samuel E. Hill ..... "The Effect of NaCl on Potentials in Nitella."
Dr. M. J. Kopac ..... "The Coalescence of a Plant Cell with Oil Drops."
Dr. Ernst Fischer ..... "The Influence of Length, Tension, and Tone upon the Birefringence of Smooth Muscles (Phascolosoma and Thyone)."
Mr. Samuel A. Corson ..... "The Mechanism of Salt Penetration in Amoeba—Some Micromanipulative Data."
Dr. Alexander Hollaender ..... "The Efficiency of Monochromatic Ultraviolet Radiation in the Activation of Arbacia Eggs."
Dr. Ethel Browne Harvey and
Dr. Alexander Hollaender ..... "Activation of Centrifuged Whole Eggs of Arbacia and Their Fractions by Monochromatic Ultraviolet Radiation."
Dr. B. R. Nebel,
Dr. E. B. Harvey and
Dr. Alexander Hollaender ..... "The Cytology of Arbacia punctulata Activated by Monochromatic Ultraviolet Radiation."
Mr. John A. Frank ..... "The Relationship of Sperm Extracts to the Fertilization Reaction in Arbacia."
Dr. L. V. Heilbrunn and
Mr. Karl M. Wilbur ..... "Stimulation and Nuclear Breakdown in the Nereis Egg."
Mr. Edward L. Chambers ..... "The Movement of the Egg Nucleus in Relation to the Sperm Aster in Echinarachnius."
Dr. Robert Chambers ..... "The Physical State of the Wall of the Furrow in a Dividing Cell."

Friday, August 27

Dr. A. Orville Dahl ..... "Chromosome Studies in Sundew (Drosera)."
Miss M. Catherine Hinchey ... "Mitosis in the Giant Amoeba, Chaos chaos Linnaeus."
Dr. James A. Miller ..... "Some Effects of Oxygen on Polarity in Tubularia crocea."
Dr. Faith Stone Miller and
Dr. James A. Miller ..... "Some Effects of Strychnine on Reconstitution of Hydranth Primordia in Tubularia crocea."
Dr. Horace W. Stunkard ..... "The Life Cycle of Moniezia expansa."

Dr. Richard G. Abell and
Dr. Eliot R. Clark ....."A New Method for Studying the pH of the Intercellular Substance in the Living Mammal."

Dr. Richard G. Abell and
Dr. Eliot R. Clark ....."The Behavior of Living Mammalian Arterioles, Capillaries, and Venules When Exposed to CO_2."

Dr. Eliot R. Clark and
Mrs. Eleanor Linton Clark ...."The Control of Peripheral Circulation in the Mammal."

Dr. Louis Loeffler ....."The Structure of the Liver Lobule."

Dr. John B. Gaylor and
Dr. Ernst Scharrer ....."A Preliminary Note on the Innervation of the Swim-bladder of the Sea-robin."

Dr. W. Gardner Lynn ....."The Origin and Development of the Thyroid in Eleutherodactylus, an Anuran with No Tadpole Stage."

Miss Virginia Mayo ....."Some Effects of Mammalian Follicle-stimulating and Luteinizing Hormones in Adult Female Urodeles."

Dr. G. H. Parker ....."The Relation of Melanophore Responses to Vascular Disturbances."

Mr. E. P. Hiatt and
Dr. J. K. W. Ferguson ....."Some Effects of Chloroform on the Respiratory Systems of Yeast."

Dr. Jean Brachet ....."The Oxygen Consumption of Activated and Fertilized Eggs of Chaetopterus."

Dr. M. E. Krahl,
Miss Anna K. Keltch and
Dr. G. H. A. Clowes ....."Influence of Respiratory Inhibitors on Stimulation of Metabolism by Nitro and Halo Phenols."

Dr. G. H. A. Clowes,
Dr. M. E. Krahl and
Miss Anna K. Keltch ....."Substituted Phenols as Inhibitants of the Fertilization of Arbacia and of Ciliary Movement of Arenicola Larvae."

Miss Anna K. Keltch,
Dr. M. E. Krahl and
Dr. G. H. A. Clowes ....."Stimulation of the Rate of Cell Division of Arbacia Eggs by Carcinogenic Hydrocarbons."

Dr. Albert Tyler and
Mr. N. H. Horowitz ....."The Molecular Species Concerned in the Action of Substituted Phenols on Marine Eggs."

Dr. Kurt C. Stern and
Dr. Kurt Salomon ..... "Ovoverdin, a Pigment Chemically Related to Visual Purple."

Mr. Louis-Paul Dugal and
Dr. Laurence Irving ..... "The Increase of CO_2 and Decalcification in Certain Pelecypods."

Dr. J. K. W. Ferguson and
Mr. E. C. Black ..... "The Effect of pH and Ionic Strength on the Activity of Carbonic Anhydrase."

Dr. J. K. W. Ferguson,
Mr. S. M. Horvath and
Dr. J. A. Pappenheimer ..... "The State of Carbon Dioxide in Dogfish Blood."

Dr. M. H. Jacobs and
Dr. A. K. Parpart ..... "The Influence of Certain Alcohols on the Permeability of the Erythrocyte."

Dr. A. K. Parpart,
Dr. M. H. Jacobs and
Mr. A. J. Dzienmian ..... "Ionic Exchanges of Erythrocytes Inferred from Volume Changes."

PAPERS READ BY TITLE

Dr. L. G. Barth ..... "Oxygen as a Controlling Factor in the Regeneration of Tubularia."

Dr. Sinisha B. Bogdanovitch ... "The Effects of Different Drugs on the Melanophores of Fundulus heteroclitus."

Dr. Sinisha B. Bogdanovitch ... "Further Investigations on the Effect of Tissue on Different Drugs."

Mr. Barry Commoner ..... "A Quantitative Study of the Staining of Marine Eggs by Neutral Red."

Dr. John A. Frisch, S.J. ..... "The Adaptation of Paramecium to Sea-water."

Dr. Charlotte Haywood and
Dr. Abby Turner ..... "The Water and Fat Content of Skeletal Muscle in Marine Fishes."

Dr. Walter N. Hess ..... "Reactions to Light of Different Intensities in Dolichoglossus kowalevskyi."

Dr. Hope Hibbard ..... "The Hatching of the Squid."

Dr. D. L. Hopkins ..... "The Vacuole System of the Marine Amoeba, Flabellula mira."

Miss Lois Hutchings ..... "Effect of Electrical Shocks upon the Division Rate of Stylonychia pustulata as Measured by the Interdivisional Period."

Dr. M. H. Jacobs and
Mr. H. N. Glassman ..... "Further Comparative Studies on the Permeability of the Erythrocyte."
Dr. J. M. Johlin ..... "The Attenuation of Toxins by Interfacial Adsorption."
Miss Elsa M. Keil and
Dr. F. J. M. Sichel ..... "The Action of Acetylcholine on the Skeletal Muscle Fibers of the Frog."
Mr. John A. Moore ..... "The Effect of Pituitary on Nuclear Changes in the Egg of the Frog."
Mr. Floyd Moser ..... "The Effect of Urea upon the Surface of Unfertilized Arbacia punctulata Eggs."
Mr. Floyd Moser ..... "The Cortical Response of Arbacia punctulata Eggs to Direct Current."
Dr. Magnus Olson ..... "The Histology of the Retractor Muscles of Thyone briareus Lesueur."
Dr. C. Ladd Prosser and
Mr. A. H. Chambers, Jr. ..... "Strength-Duration Curves of Nerve Fibers in the Squid."
Dr. Leonard P. Sayles ..... "New Structures Induced by Implants of Adult Nerve Cord in the Polychaete, Clymenella torquata."
Dr. A. A. Schaeffer ..... "Cytoplasmic Division in Type B of the Giant Amoeba Chaos chaos Linnaeus."
Dr. Ernst Scharrer ..... "The Sense of Taste in the Free Fin Rays of the Sea Robin (Prionotus)."
Dr. Victor Schechter ..... "Calcium and Magnesium in Relation to Longevity of Mactra, Nereis and Hydroides Egg Cells."
Dr. F. O. Schmitt and
Dr. Otto H. Schmitt ..... "A Convenient Method for the Measurement of Nerve Respiration."
Dr. F. J. M. Sichel and
Dr. C. Ladd Prosser ..... "Temporal Relations in the Excitation of the Isolated Muscle Fiber."
Dr. H. Burr Steinbach ..... "Electrolytes in Phascolosoma Muscle."
Dr. Harry N. Stoudt ..... "Leaf Development and Vegetative Propagation in Polystichum plaschnickianum."
Dr. T. Terni ..... "Morphological and Experimental Cytology of Lobster Spermatozoa."
Dr. Abby H. Turner and
Dr. Charlotte Haywood ..... "Observations on Arterial Pressure in Marine Fishes."

Dr. Albert Tyler,
Miss Nelda Ricci and
Mr. N. H. Horowitz ..... "Respiratory Rate and Length of Fertilizable Life of Unfertilized Arbacia Eggs under Sterile and Non-sterile Conditions."

Dr. A. J. Waterman ..... "The Effect upon Gastrulation and Differentiation in Arbacia of $NiCl_2$, $CuCl_2$, and Na_2SiO_3 in Modified Artificial Sea Water."

Dr. Ralph Wichterman ..... "Studies on Living Conjugants of Paramecium caudatum."

Dr. Ralph Wichterman ..... "Conjugation in Paramecium trichium Stokes (Protozoa, Ciliata) with Special Reference to the Nuclear Phenomena."

Dr. Opal M. Wolf ..... "Mitotic Activity of Stimulated Rat Adrenals Measured by Colchicine Technique."

Dr. Opal M. Wolf ..... "Induced Breeding Reactions in Isolated Male Frogs, Rana pipiens Schreber."

DEMONSTRATIONS

Thursday, August 26

Dr. S. E. Pond and
Mr. L. F. Boss ..... "Sources of Working Current for a Potentiometer."

Dr. R. Chambers and
R. B. W. Zweifach ..... "Micro-manipulative Studies of Blood Capillaries."

Dr. J. E. Kindred ..... "Stages in the Development of Lymph Nodules."

Dr. E. A. Wolf ..... "Individual Variations in Salivary Amylase in Man."

Dr. K. S. Cole,
Dr. H. J. Curtis and
Mr. J. M. Spencer ..... "Alternating Current Wheatstone Bridge for Impedance Measurements at Frequencies from 30 to 10,000,000 Cycles."

Dr. E. R. Clark and
Mrs. Eleanor L. Clark ..... "Control of the Peripheral Circulation as Seen in the Living Mammal."

Dr. R. G. Abell and
Dr. E. R. Clark ..... "A New Method for Studying the pH of the Intercellular Substance in the Living Mammal."

Dr. V. Hamburger ..... "Limb-bud Transplantation in Chick Embryos."

Mr. J. A. Smith ..... "Types of Hermaphroditism in Mollusca."

Dr. F. O. Schmitt and
Dr. O. H. Schmitt ..... "A Convenient Method for the Measurement of Nerve Respiration."

Dr. F. O. Schmitt,
Dr. O. H. Schmitt and
Dr. R. S. Bear ..... "Action Potentials from the Giant Axon of the Squid, Loligo pealii."

Dr. S. E. Hill ..... "Action Currents in Nitella."

Dr. T. M. Sonneborn ..... "Sexuality in Paramecium."

Dr. J. B. Gaylor ..... "Innervation of the Swim-bladder of the Sea-robin."

Dr. E. Fischer ..... "Volume Changes of Muscle on Contraction."

Dr. C. E. McClung ..... "Relation of the Apical Cell to the Germ Cell in the Grasshopper Testis."

Dr. H. Irene Corey ..... "Polar Elements of Orthopteran Chromosomes."

Dr. D. H. Wenrich ..... "Mitosis in Dientamoeba fragilis."

Dr. C. L. Parmenter ..... (a) "Haploid, Diploid, and Triploid Mitoses in Parthenogenetic Frog Tadpoles."

(b) "Mono, Di, Tri, and Tetra-nucleate Blood Cells in Haploid Parthenogenetic Frog Tadpoles."

Dr. A. A. Schaeffer ..... "Types A and B of Chaos chaos Linnaeus, the Large Multinucleate Amoeba of Roesel."

Dr. A. K. Parpart ..... (a) "Method for the Rapid and Complete Separation of Erythrocytes from a Surrounding Medium."

(b) "Volume Changes of Erythrocytes Produced by an Ionic Exchange."

Dr. G. Failla ..... "New X-ray Equipment of the Marine Biological Laboratory."

Dr. T. Terni ..... (a) "Morphological and Experimental Cytology of Lobster Spermatozoa."

(b) "The Nervous System of Dogfish Embryos."

Mr. L. F. Boss and
Dr. S. E. Pond ..... "Combination Thermostat, Heat Interchanger and Relay Unit for Constant Temperature Bath."

Mr. C. G. Grand ..... "Cytolytic Effects on Marine Eggs after Puncturing with Microneedles."

Dr. M. J. Kopac	(a) "Living Valonia Aplanospores."
	(b) "Apparatus for Delivering Oil Drops under Constant Pressure."
Dr. Eleanor Carothers	(a) "Development of Mid-gut of Melanoplus differentialis."
	(b) "Effects of X-rays on Grasshopper Embryos."
Dr. A. Hollaender	"Equipment for Work with Measured Quantities of Monochromatic Radiation in the Visible and Ultraviolet."
Mr. L. F. Boss, Dr. A. Hollaender and Dr. S. E. Pond	"Inexpensive 500 Volt D.C. Power-supply for High Intensity Ultraviolet."
Dr. B. R. Nebel and Dr. E. B. Harvey	"Cytological Observations on Arbacia Eggs Activated by Monochromatic Ultra-violet Radiation."
Dr. L. Loeffler	"Injected Liver of Toadfish."
Dr. R. W. Root and Dr. W. Etkin	"Use of the Constant Flow Method for Serial Determinations of Oxygen Consumption in Toadfish."

8. MEMBERS OF THE CORPORATION

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Maryland.
Billings, Mr. R. C., 66 Franklin St., Boston, Massachusetts.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Massachusetts.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Massachusetts.
Jackson, Miss M. C., 88 Marlboro St., Boston, Massachusetts.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Massachusetts.
Kidder, Mr. Nathaniel T., Milton, Massachusetts.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City.
Lee, Prof. F. S., College of Physicians and Surgeons, New York City.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City.

MORGAN, PROF. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, California.
MORGAN, MRS. T. H., Pasadena, California.
MORRILL, DR. A. D., Hamilton College, Clinton, N. Y.
NOYES, MISS EVA J.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
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SHEDD, MR. E. A.
THORNDIKE, DR. EDWARD L., Teachers College, Columbia University, New York City.
TREADWELL, PROF. A. L., Vassar College, Poughkeepsie, N. Y.
TRELEASE, PROF. WILLIAM, University of Illinois, Urbana, Illinois.
WILSON, DR. E. B., Columbia University, New York City.

2. REGULAR MEMBERS, 1937

ADAMS, DR. A. ELIZABETH, Mount Holyoke College, South Hadley, Massachusetts.
ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
ADOLPH, DR. EDWARD F., University of Rochester Medical School, Rochester, New York.
ALLEE, DR. W. C., The University of Chicago, Chicago, Illinois.
ALLYN, DR. HARRIET M., Mount Holyoke College, South Hadley, Massachusetts.
AMBERSON, DR. WILLIAM R., Department of Physiology, University of Maryland, School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.
ANDERSON, DR. E. G., California Institute of Technology, Pasadena, California.
ARMSTRONG, DR. PHILIP B., University of Alabama, Medical School, Tuscaloosa, Alabama.
AUSTIN, DR. MARY L., Wellesley College, Wellesley, Massachusetts.
BAITSELL, DR. GEORGE A., Yale University, New Haven, Connecticut.
BAKER, DR. H. B., University of Pennsylvania, Philadelphia, Pennsylvania.
BALDWIN, DR. F. M., University of Southern California, Los Angeles, California.
BALL, DR. ERIC G., Johns Hopkins Medical School, Baltimore, Maryland.
BALLARD, DR. WILLIAM W., Dartmouth College, Hanover, New Hampshire.

BARD, PROF. PHILIP, Johns Hopkins Medical School, Baltimore, Maryland.

BARRON, DR. E. S. GUZMAN, Department of Medicine, The University of Chicago, Chicago, Illinois.

BARTH, DR. L. G., Department of Zoölogy, Columbia University, New York City.

BEADLE, DR. G. W., School of Biological Sciences, Stanford University, California.

BECKWITH, DR. CORA J., Vassar College, Poughkeepsie, New York.

BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, Louisiana.

BENNITT, DR. RUDOLF, University of Missouri, Columbia, Missouri.

BIGELOW, DR. H. B., Museum of Comparative Zoölogy, Cambridge, Massachusetts.

BIGELOW, PROF. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

BINFORD, PROF. RAYMOND, Guilford College, Guilford College, North Carolina.

BISSONNETTE, DR. T. HUME, Trinity College, Hartford, Connecticut.

BLANCHARD, PROF. KENNETH C., Washington Square College, New York University, New York City.

BODINE, DR. J. H., Department of Zoölogy, State University of Iowa, Iowa City, Iowa.

BORING, DR. ALICE M., Yenching University, Peking, China.

BOZLER, DR. EMIL, Ohio State University, Columbus, Ohio.

BRADLEY, PROF. HAROLD C., University of Wisconsin, Madison, Wisconsin.

BRIDGES, DR. CALVIN B., California Institute of Technology, Pasadena, California.

BRONFENBRENNER, DR. JACQUES J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.

BRONK, DR. D. W., University of Pennsylvania, Philadelphia, Pennsylvania.

BROOKS, DR. S. C., University of California, Berkeley, California.

BROWN, DR. DUGALD E. S., New York University, College of Medicine, New York City.

BUCKINGHAM, MISS EDITH N., Sudbury, Massachusetts.

BUDINGTON, PROF. R. A., Oberlin College, Oberlin, Ohio.

BULLINGTON, DR. W. E., Randolph-Macon College, Ashland, Virginia.

BUMPUS, PROF. H. C., Duxbury, Massachusetts.

BYRNES, DR. ESTHER E., 1803 North Camac Street, Philadelphia, Pennsylvania.

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CALVERT, PROF. PHILIP P., University of Pennsylvania, Philadelphia, Pennsylvania.
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CARVER, PROF. GAIL L., Mercer University, Macon, Georgia.
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CATTELL, PROF. J. MCKEEN, Garrison-on-Hudson, New York.
CATTELL, MR. WARE, Garrison-on-Hudson, New York.
CHAMBERS, DR. ROBERT, Washington Square College, New York University, Washington Square, New York City.
CHENEY, DR. RALPH H., Biology Department, Long Island University, Brooklyn, New York.
CHIDESTER, PROF. F. E., Auburndale, Massachusetts.
CHILD, PROF. C. M., Jordan Hall, Stanford University, California.
CLARK, PROF. E. R., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
CLARK, DR. LEONARD B., Union College, Schenectady, New York.
CLELAND, PROF. RALPH E., Goucher College, Baltimore, Maryland.
CLOWES, DR. G. H. A., Eli Lilly and Co., Indianapolis, Indiana.
COE, PROF. W. R., Yale University, New Haven, Connecticut.
COHN, DR. EDWIN J., 183 Brattle Street, Cambridge, Massachusetts.
COLE, DR. ELBERT C., Department of Biology, Williams College, Williamstown, Massachusetts.
COLE, DR. KENNETH C., College of Physicians and Surgeons, Columbia University, 630 W. 168th Street, New York City.
COLE, DR. LEON J., College of Agriculture, Madison, Wisconsin.
COLLETT, DR. MARY E., Western Reserve University, Cleveland, Ohio.
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COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Maine.
COSTELLO, DR. DONALD P., Department of Zoölogy, University of North Carolina, Chapel Hill, North Carolina.

COSTELLO, DR. HELEN MILLER, Department of Zoölogy, University of North Carolina, Chapel Hill, North Carolina.

COWDRY, DR. E. V., Washington University, St. Louis, Missouri.

CRAMPTON, PROF. H. E., Barnard College, Columbia University, New York City.

CRANE, MRS. C. R., Woods Hole, Massachusetts.

CROWELL, DR. P. S., JR., Department of Zoölogy, Miami University, Oxford, Ohio.

CURTIS, DR. MAYNIE R., Crocker Laboratory, Columbia University, New York City.

CURTIS, PROF. W. C., University of Missouri, Columbia, Missouri.

DAN, DR. KATSUMA, Misaki Biological Station, Misaki, Japan.

DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Virginia.

DAWSON, DR. A. B., Harvard University, Cambridge, Massachusetts.

DAWSON, DR. J. A., The College of the City of New York, New York City.

DEDERER, DR. PAULINE H., Connecticut College, New London, Connecticut.

DILLER, DR. WILLIAM F., Dartmouth College, Hanover, New Hampshire.

DODDS, PROF. G. S., Medical School, University of West Virginia, Morgantown, West Virginia.

DOLLEY, PROF. WILLIAM L., University of Buffalo, Buffalo, New York.

DONALDSON, PROF. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.

DONALDSON, DR. JOHN C., University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.

DUBOIS, DR. EUGENE F., Cornell University Medical College, 1300 York Avenue, New York City.

DUGGAR, DR. BENJAMIN M., University of Wisconsin, Madison, Wisconsin.

DUNGAY, DR. NEIL S., Carleton College, Northfield, Minnesota.

DURYEE, DR. WILLIAM R., Department of Biology, Washington Square College, New York University, New York City.

EDWARDS, DR. D. J., Cornell University Medical College, 1300 York Avenue, New York City.

ELLIS, DR. F. W., Monson, Massachusetts.

FAURÉ-FREMIET, PROF. EMMANUEL, Collège de France, Paris, France.

FERGUSON, DR. JAMES K. W., Department of Physiology, Ohio State University, Columbus, Ohio.

FISCHER, DR. ERNST, Department of Physiology, Medical College of Virginia, Richmond, Virginia.

FISHER, DR. KENNETH C., Department of Biology, University of Toronto, Toronto, Canada.

FLEISHER, DR. MOYER S., School of Medicine, St. Louis University, St. Louis, Missouri.

FORBES, DR. ALEXANDER, Harvard University Medical School, Boston, Massachusetts.

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THE REPRODUCTIVE CYCLE OF BRACHYRAPHIS EPISCOPI, AN OVOVIVIPAROUS PŒCILIID FISH, IN THE NATURAL TROPICAL HABITAT

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Introduction

Reproduction in pœciliid fishes occurs in short recurring cycles. Field studies on the reproduction of these fishes, principally *Gambusia affinis* and *Heterandria formosa*, in the temperate and subtropical zones, have demonstrated that reproduction reaches its peak in spring and early summer, declines during late summer and fall, ceases altogether during the winter and begins again in early spring. Laboratory studies have shown that reproduction is continued longer in the fall and begins earlier in the spring when the gravid females are kept under constant conditions as regards temperature and food. The decline in the reproductive period would be associated with other factors, either genetic or some uncontrolled environmental factor. In a laboratory study of various pœciliids, principally *Xiphophorus helleri*, *Lebistes reticulatus*, *Pœcilistes pleurospilus* and *Heterandria formosa*, the writer (Turner, 1937) came to the conclusion that the seasonal variation of light was a factor in controlling the reproductive cycle, probably through the action of the pituitary. Hoover and Hubbard (1937) have produced experimental evidence that exposing maturing brook trout to periods of light in excess of those of seasonal daylight will induce early maturing and spawning.

In order to test the possibility of light as one of the controlling factors in the reproductive cycles in pœciliids, one might artificially control the light exposure so that it would be uniform throughout the year and equal daily to that under which the fish would reproduce freely. Or, a study might be made of the reproduction of a pœciliid fish under natural tropical conditions in which the daily light exposure is fairly uniform and in a selected habitat in which food and temperature change but little during the annual period.

This paper is written to record the results of a study of reproduction in *Brachyrhaphis episcopi*, a pœciliid fish which is common in a small

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stream near the laboratory at Barro Colorado Island in the Panama Canal Zone about nine degrees north of the equator. The fish was chosen because it is a typical pœciliid, was easily accessible, and occurred in sufficient numbers during all seasons of the year to make a critical study feasible. Collections were made during late June and early July in the summer of 1936 with a wide silk bolting cloth dip net in such a way as to secure a fair sample of all sizes of *Brachyrhaphis* in the stream. An employee of the laboratory was instructed in the manner of collecting and through the kindness of Mr. James Zetek, the collections were shipped to the writer. The specimens were fixed in formalin and transferred to 70 per cent alcohol. Collections representing all sizes obtainable were obtained for each month except May.

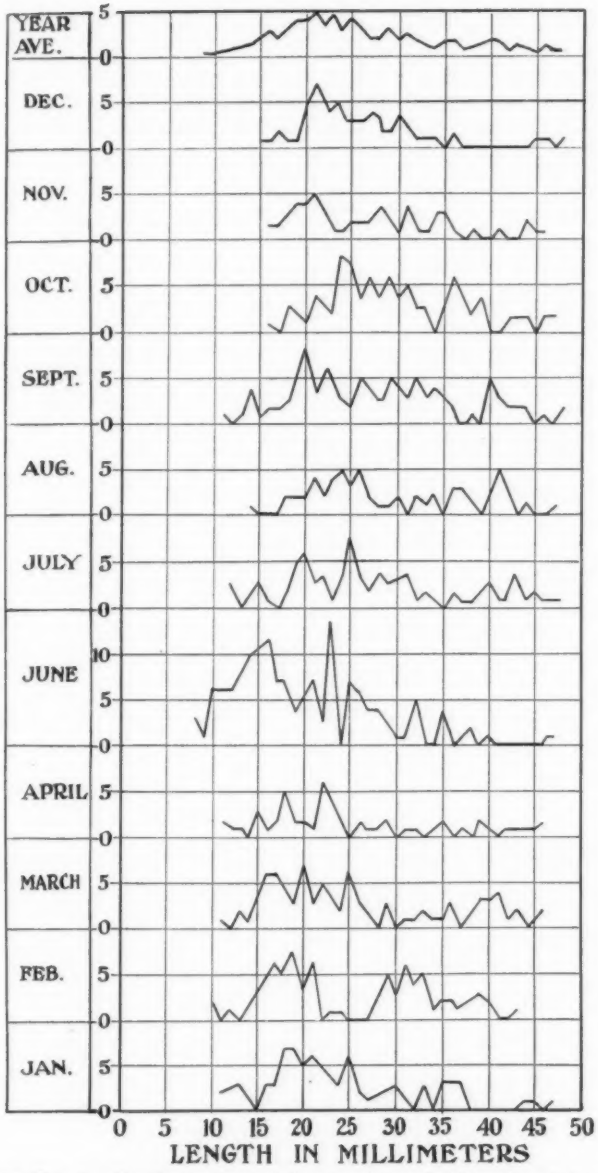
In all, 1,139 immature and female fishes were collected and measured and 442 were dissected for the purpose of counting and staging the embryos and measuring the developing ovocytes. A total of 288 mature or partially mature males were examined.

Allee (1926) made a careful study of the variations in temperature, rainfall and other environmental features at this locality and reported that the seasonal variation in temperature on the jungle floor through which this stream flows is slight. During the rainy season there is an abundance of water in the stream but during the dry season the stream is reduced to a series of connected pools. At no time is the water in the pools more than two feet deep. Floating debris, insect adults and larvæ and other small animals upon which the fish feeds are abundant. The daily duration of daylight varies from eleven hours and thirty-six minutes in late December to twelve hours and forty minutes in late June. This natural habitat in general has little variation in food supply, temperature and light.

Lengths of Fishes in Monthly Collections

Some information may be obtained by taking the lengths of all the fishes obtained in each month and comparing them throughout the year. Such a record is shown in Fig. 1. If a seasonal breeding cycle exists it might be expected that the minimal size would occur at the beginning of the breeding season and that the minimal size would increase as growth takes place. If the results are plotted graphically it might be expected also that a mode for the entire population would gradually move to the right as all fishes continued to grow.

It will be noted that the point for the minimal length, representing newly born fishes, does not move to the right. Specimens between 8 and 17 mm. in length are present in each month. It is believed that the minimal size represented in June is more characteristic than that



TEXT FIG. 1. Graph showing relative numbers of fishes of each length for each month. Numbers in each month are shown vertically, lengths of specimens in millimeters horizontally.

obtained for other months. The writer made the June collection and took great pains to secure the smallest fishes in the stream. The other collections were made by the caretaker at the laboratory and it is likely that the smallest specimens were overlooked, especially since they have a tendency to hide in the vegetation at the stream margin. The evidence indicates that young are being produced in each month of the year.

If a graph is plotted based upon the entire annual collection instead of the monthly collections, the mode for the entire population will appear in lengths between 20 and 30 mm. When the monthly curves are smoothed it will appear that the largest number occurs in each month at the same length. Since the mode shows no tendency to move to the right during any season it is assumed that it is maintained in its position by a continual new supply of young.

As far as this evidence goes it indicates that there is no season relation in the production of young and that the fall decline and winter cessation in reproduction found in the pœciliids kept in laboratories at a latitude of about forty degrees are lacking.

Brood Size in Different Months and Seasonal Occurrence of Gravid Females

If reproduction is seasonal it would be expected that during some months gravid females would be lacking altogether or that a smaller proportion of females would be carrying broods. Except in the smallest of the mature females no such relation was found. Gravid females occurred in each month and in about the same proportion. An occasional female from 24 to 26 mm. in length is found to be carrying young but the data are too scanty for any conclusion concerning a seasonal relation to time of maturity.

In some pœciliids studied in the laboratory (Turner, 1937) not only does the interval between broods become shorter as reproduction approaches its height but there is an increase in the number of embryos in succeeding broods. With this in mind, all the gravid females of *Brachyrhaphis* obtained in each month were dissected to determine whether the number of embryos in each brood varied seasonally. Monthly averages of brood size for each length of gravid female are indicated in Table I. The data show that: (1) There is considerable variation in brood size for each length of gravid female. (2) There is no seasonal trend for size of broods in gravid females of any length.

The conclusion from this evidence indicates again that reproduction is continual throughout the year and that no seasonal increase or decrease in reproductive activity exists.

Incidental information is obtained concerning the fecundity of

females of different lengths and of the relative contribution to the population of females of different lengths by considering brood size for each length and the relative abundance in the population as a whole of females of different lengths (Table III). It will be noted that brood size is very small in 25 to 30-mm. females and that it rises to a maxi-

TABLE I

Table indicating brood size in gravid females of different lengths for each month

Length of gravid female in mm.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Yearly average for each length
24													
25	1												1
26	2.5			3									2.9
27	4	2	3									4	3.25
28	6			1						3		3	3.25
29	9	1	1.5	1					2			4.6	3.18
30	6		8	6.5					3		5	3	5.25
31	6			3		6		4	3	3	4	5.5	4.3
32	7					4				4	5		5
33	10	7		5		7	7			5	6.5		6.9
34		7	7			11	10					6.5	8.3
35	8.6			8		8	9				5.5		8.4
36	17.5	9	5.5			9	9	5		3		14	9
37	6		5			5.5	9.5	6		7			6.5
38			8	8.6		19.5	18.5	8.5	3	8			10.6
39			11.5	12		12.6	9.7		5.5	10.5			10.3
40		8	8	9			10	6	12.5				8.9
41		8		11			11.5	9.5	12		4		9.6
42			13.5	18			28	9	5		19		15.4
43						25.5	14	18	11	10		30	19.1
44		30	14				13	15	8	18.5	22		17.2
45	28	41					18	15		13	23	9	21
46			23			35				20		20	23.6
47	46	30	17					21	29	24			27.8
48									13			40	26.5
Average for all lengths per month	11.3	14.3	9.6	7.2		12.7	12.2	10.3	9.2	9.9	10.4	8.9	

mum in the largest females. The total production of young by the largest females is not greater, however, than that of females somewhat smaller because of their relatively smaller numbers in the population as a whole.

RELATION OF OVOCYTE DEVELOPMENT TO EMBRYO STAGES

In a study of the relation of ovocyte development in gravid ovaries to stages of embryos in the same ovaries (Turner, 1937), it was dis-

covered that different relationships exist in different groups of pœciliids. In *Gambusia* the ovocytes of a gravid ovary remain small until the brood of embryos in the ovary is evacuated. In *Xiphophorus, Lebistes* and some other species the ovocytes increase in size while the ovary is gravid and in *Quintana atrizona* the ovocytes have grown to full size and are ready for fertilization immediately after the evacuation of a brood. In *Pœcilistes pleurospilus, Pœciliopsis infans* and *Heterandria formosa,* the ovocytes have grown not only to maximal size but fertilization has taken place before the older brood of embryos is evacuated and two or more broods of embryos are found in the ovary at the same time. The condition described above occurs in laboratory-reared specimens at the height of the reproductive season. When reproduction is at a low ebb the ovocytes grow slowly and are rela-

TABLE II

Table showing stages of developing ovocytes for each stage of developing embryo in the same gravid ovary for each month

Stages of embryos in gravid ovaries	Developing ovocytes: diameter in mm.												
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Stage 0.....	.38	.28	.23	.21		.26	.28	.34	.29	.19	.28	.20	.27
Stage No. 1		.3				.25	.2					.22	.24
Stage No. 2	.27		.13	.25		.21	.2		.24	.15	.25	.26	.22
Stage No. 3	.46	.25	.20	.25		.29	.29	.22	.23	.21	.24	.24	.26
Stage No. 4	.50	.56	.27	.64		.72	.51	.35	.55	.33	1.05	.64	.55
Stage No. 5	1.28	1.05	.86	1.41		.90	1.3	.98	1.40	1.03	1.39	1.37	1.18

tively much smaller at the time of the birth of the embryos than they are when reproduction is at its height.

This latter fact is used as a third method of searching the material for evidence of a seasonal variation in reproduction. If a seasonal high and low phase of reproduction exists the growing ovocytes should be smaller at the low phase and larger at the high phase provided that they were referred to some fixed stage in reproduction such as the birth of the embryos or to some definite stage of development in the embryos of a brood. Gravid females were dissected and the embryos in the ovary were staged (Table II). Stage 0 represents a condition in the ovary in which no embryos are present and the ova which are to form the next brood are full-sized but not yet fertilized. Stage 5 represents a condition in which the embryos are ready for birth and stages 1, 2, 3 and 4 represent conditions in which the embryos are in intermediate stages. At any stage except 0 there is a group of de-

veloping ovocytes definitely advanced over younger stages and about equal in number to the embryos in the brood held in the same ovary. These developing ovocytes vary somewhat in diameter so that it was necessary to take an average diameter.

The findings are shown in tabular form in Table II. A monthly average of the diameters of developing ovocytes is shown for each embryo stage in the broods held in the ovaries. A yearly average is also

TABLE III

Table indicating productivity of females of different lengths

Length in mm.	No. of gravid females; yearly average for each length	Average no. of young per gravid ovary	Relative productivity
25	4	1	4
26	3.2	2.9	7.9
27	2.4	3.25	7.8
28	2.5	3.25	8.17
29	3.2	3.18	10.17
30	2.3	5.25	12.07
31	2.6	4.3	11.18
32	2.2	5	11
33	2	6.9	13.8
34	1.2	8.3	9.9
35	2	8.4	16.8
36	2	9	18
37	1.2	6.5	7.8
38	1.3	10.6	13.9
39	1.5	10.3	15.5
40	1.7	8.9	15.1
41	1.5	9.6	14.4
42	1	15.4	15.4
43	1.2	19.1	23.0
44	1	17.2	17.2
45	.6	21	12.6
46	1	23.6	23.6
47	.6	27.8	16.6
48	.5	26.5	13.2

shown. In general it will be noted that the ovocytes do not increase in size during stages 0, 1, 2 and 3 but increase over four-fold during stages 4 and 5. A comparison of the monthly averages with the yearly averages indicates that there is considerable variation, probably due to the small numbers of specimens examined, in the diameter of the ovocytes from month to month, but that no definite trend is shown toward a seasonal increase or decrease.

Age of Maturity in Males

In the males of pœciliid fishes maturity is marked externally by the development of a gonopod, formed by a metamorphosis of the anal fin. The testis develops and spermatogenesis begins at the same time or a little earlier. Once maturity has been attained the production of spermatozoa seems to be continual. Males will reproduce during any season in the laboratory and attempts to find seasonal waves either in spermatogenesis or in reproductive behavior have failed. Any study of the testis in *Brachyrhaphis* for evidence for a seasonal variation in maturation stages in adults would not be expected to yield results and no such study was attempted.

A study was made of the size at which maturity is indicated by the development of the gonopod and of the relative numbers from month to month of the mature males and of males with partially metamorphosed anal fins. The results are summarized as follows: (1) Some males mature at an early stage and have fully metamorphosed gonopods when they are 15 mm. in length. (2) Some males postpone maturity and specimens with partially metamorphosed gonopods may be found at any length up to 36 mm. Specimens with transforming gonopods are most abundant at lengths from 16 to 25 mm. (3) Specimens of 15 mm. with gonopods fully developed and larger specimens with developing gonopods were found in each month in which material was obtained.

These findings indicate that there is no seasonal variation in the production of maturity in males.

Discussion and Summary

Three lines of approach are used in the study of females and immature specimens to determine whether there exists in *Brachyrhaphis episcopi*, living in a natural tropical habitat, a seasonal variation in reproduction such as exists in pœciliid fishes living in natural habitats in sub-tropical and temperate zones and in laboratory-reared specimens maintained in the temperate zone. It is concluded that reproduction occurs in the short cycles characteristic of pœciliid fishes but that the cycles are continuous and are not seasonal for the following reasons: (1) Young are produced at about the same rate in each month of the year as indicated by measurements of lengths of all specimens taken in each month. (2) Gravid females are taken in each month, indicating that fertilization takes place the year round. (3) The relative proportion of gravid to non-gravid females in each month is approximately equal. (4) The age, as indicated by length of specimens at which females become mature, shows no significant seasonal variation.

(5) The average number of embryos in a brood for each size of female shows little seasonal variation. (6) In the same gravid ovary the relative interval between embryos in definite stages and the stage of development of developing ovocytes does not vary seasonally.

A study of the age of maturity of males and of the relative abundance of mature and partially mature males during the different seasons indicates also that no seasonal variation exists.

In temperate and sub-tropical zones in natural habitats the recurring reproductive cycles in pœciliid fishes decline in late summer and fall and cease altogether in the winter. Here temperature is obviously one factor which is related to reproduction. It has been shown, however (Barney and Anson, 1921*b*), that a decline in reproduction begins while the temperature of the water is still favorable for further reproduction. In pœciliids maintained in the laboratory with food and temperature controlled but with the exposure to light varying with the seasonal fluctuation in daylight, reproduction rises with the approach of spring and wanes during late summer. It is assumed that light is also a factor in the control of reproduction and that the rather uniform seasonal duration of daylight at the latitude of the Panama Canal Zone (9° N.) is concerned in the maintenance of a continual reproduction in *Brachyrhaphis*. The minimal duration of daylight here at 9° N. is eleven hours and thirty-six minutes and is equivalent to the duration of light on March 7 at a latitude of 42° N (Chicago latitude). The maximal duration at 9° N. is twelve hours and thirty-nine minutes and is equivalent to that on April 1 at a latitude of 42° N. If duration of daylight is concerned as a factor partially controlling reproduction, it must be concluded that for *Brachyrhaphis* at least the excess of daylight in late spring and early summer at 42° N. latitude would be unnecessary for the maintenance of reproduction and the question naturally arises as to the effect of this excess of daylight on the reproduction of fishes which occur in natural habitats at 42° N. latitude. It will be interesting to determine whether pœciliids other than *Brachyrhaphis* will reproduce continually when placed under experimental conditions with food and temperature controlled and with exposure to light varying within the limits of duration and intensity occurring at 9° N. latitude.

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THE SEASONAL CYCLE IN THE GONADS OF FUNDULUS

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From the voluminous work on mammals, birds, and amphibians and to a less extent on reptiles also, the factors concerned in the control of the sex cycle in these forms are becoming more clearly defined. Fishes, however, have received little attention in this respect. Experiments designed to investigate such factors as might be involved in controlling the annual sex cycle which *Fundulus heteroclitus* exhibits were begun some time ago. When an attempt was made to analyze the results of these experiments it was clear at once that before any orderly arrangement of data could be made it would be necessary to determine the condition of the gonads at intervals during the normal seasonal cycle. Accordingly normal individuals were collected at regular intervals over a period of fifteen months, for the most part from the Delaware River near Palmyra, New Jersey, though animals were obtained at Woods Hole during June, July and August. These animals were killed either immediately or within a week after collection, the animals and gonads were weighed, and the gonads, pituitary and thyroid glands were preserved for sectioning. The following is a report on the seasonal variation in the gonads of these animals.

The male *Fundulus* is provided with a pair of testes, elongate organs lying one on either side of the mid-line, closely pressed against the ventral wall of the swim bladder to which they are attached by short mesorchia (Fig. 1). In general the two testes are separated for the greater part of their length by a mesentery which supports the digestive tract, but they may be joined to an extent which varies in different individuals. A short duct leaves the posterior end of each testis, joins the corresponding duct from the opposite side and runs to the genital pore which, in the male, is at the tip of a short, conical elevation at the base of the anal fin.

Each testis has a thin fibrous capsule which is provided with a mesothelial covering. Beneath the capsule a central medulla and a peripheral cortex can be distinguished (Fig. 2). The medulla is made up of fibrous connective tissue in which are embedded many sperm

[1] The early work on this material was carried out at the Department of Anatomy, University of Pennsylvania.

ducts, blood vessels and clusters of peculiar cells which will be described below. The cortex is divided into somewhat indistinct lobules by small amounts of connective tissue, each lobule containing several cysts which are closely packed with spermatogenic cells. In these sections, which were not prepared for special cytological details, only four types of spermatogenic cells could be distinguished. The largest of these, cells with large nuclei and one or two prominent nucleoli, are similar to the primary spermatogonia described by Turner (1919) in the perch and by Foley (1926) in *Umbra*. The next largest cell, about two-thirds the size of the primary spermatogonia, is one in which the nucleus occupies a much larger percentage of the cytoplasm. These are the most actively dividing cells of the testis. They must include secondary spermatogonia as well as both primary and secondary spermatocytes but these three cell types could not be distinguished with accuracy. The third kind of cell observed is somewhat smaller with a deep staining nucleus and is undoubtedly the spermatid. Finally spermatozoa with their rounded heads and elongate tails can be readily distinguished. In any one cyst all the cells are alike.

The seasonal cycle observed in the testes of these animals is as follows. In late May, June, and July the medulla is swollen with ducts filled with sperm (Fig. 3). Nearly all the cysts of the cortex are also filled with sperm but around the periphery there is a narrow zone composed mainly of primary spermatogonia. These cells are dividing rapidly, producing small cysts of secondary spermatogonia. Thus before the matured sperm of one generation of sex cells is completely expelled the beginnings of the next generation are apparent. Ordinarily the sperm are shed during June and July. In some cases, however, the sperm are not expelled until the last of August. Complete expulsion of the sperm is rare. Small clusters of sperm which are apparently left from the previous period of sexual activity can be found in nearly every testis examined regardless of the time of year. After the spawning season there is a striking reduction in the amount of cortical tissue relative to the total diameter of the testis, even though the preliminary stages in the production of the next generation of sperm have already produced small cysts (Fig. 4). Cells within these cysts continue to divide slowly, the cysts gradually enlarge, and the cortex of the entire testis increases in thickness. In September a few spermatids appear. Throughout the period from October to the latter part of February there is a gradual increase in the number of spermatogonia, spermatocytes, and spermatids, and the cysts become larger. Sometime in March the cells within the cysts begin to divide more rapidly, as evidenced by the number of mitotic figures and the

rapid increase in size of the cysts during this period, and spermatids become increasingly more numerous in proportion to other cells. In late April and in May spermatozoa appear in increasingly large numbers.

During this process the testis changes markedly in color and size. The active testis found during May, June and the early part of July is

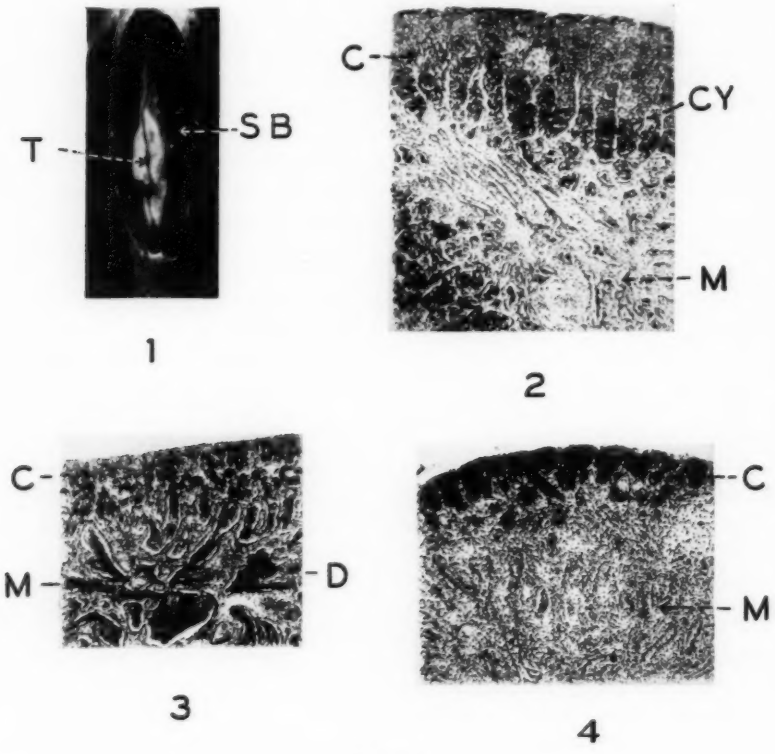

PLATE I

FIG. 1. Male *Fundulus*, digestive organs removed. × 1.5. *SB*, artificial opening in ventral wall of swim-bladder to show its relation to the testis; *T*, testis.

FIG. 2. Cross-section of testis of animal killed 12/4/35. × 80. *C*, cortex; *CY*, cysts of spermatogenic cells; *M*, medulla.

FIG. 3. Cross-section of "ripe" testis of animal killed 7/30/35. × 80. *C*, cortex; *D*, sperm duct filled with sperm; *M*, medulla.

FIG. 4. Cross-section of exhausted testis of animal killed 7/30/35. × 80. *C*, cortex; *M*, medulla.

milky white and definitely enlarged. The exhausted testis in late July and August is colorless for the most part, more or less transparent and much reduced in size. Figure 5 indicates the seasonal changes in weight of the testis relative to the total body weight. This chart is based on data from 58 animals collected over a period of fifteen months, from December 1935 to March 1937 inclusive, the average weights for the calendar months being taken. While the number of animals on which this chart is based is insufficient for accurate statistical analysis it is apparent that the testis forms the greatest percentage of the total body weight during the period from late in March to the latter part of June. When the averages for the months of January, February and March, 1937, are separated from those of the same months of the preceding year and the curve is drawn for a period of fifteen months the differences between such a curve and the one shown are slight and are probably due to the small number of animals which could be collected during this period of the two years. Such a curve also shows a rise in weight of the testis during March of both years. Sections of the testes of these animals show that the greatest weight of the testis corresponds to that period in the seasonal cycle from the beginning of rapid division of spermatogenic cells and the consequent enlargement of the cysts that occurs in March up to the time when the majority of the sperm is expelled, usually in June or July. This curve for the proportion of the body weight formed by the testis in *Fundulus* at different periods throughout the year is somewhat different from a similar curve for the perch (Turner, 1919). Although in this latter form the spawning season is only a month or so earlier than that in *Fundulus*, the sudden increase in weight of the perch testis appears at the end of the summer, notably earlier than it occurs in *Fundulus*.

In the medulla of the testis clusters of peculiar cells which might be considered interstitial cells were found. Much has been written concerning the nature, origin, and function of interstitial tissue in teleosts. They are apparently absent in many teleosts (Kolmer and Scheminzky, 1922), present in others (Stieve, 1933). Courrier (1921) believes they arise from leucocytes that migrate into the testis, and that they are responsible for the development of such secondary sex characters as the color patterns in males. Van Oordt (1925), on the other hand, thinks it is improbable that there is in the fish testis a group of cells which are a specific source for sex hormones. In *Fundulus*, after the sperm are shed late in July, there are present in the medulla of the testis numerous clusters of cells with shrunken, pycnotic nuclei. The cytoplasm of these cells stains pale yellow and frequently has a large vacuole (Fig. 6). These cell clusters are most conspicuous

just after the sperm are shed but they persist for long periods and can be found in all the testes examined except in those fixed either immediately before or during the spawning season, when the connective tissue in the medullary region is reduced to narrow strands by the large number of spermatogenic cells present. The origin of these cells is doubtful. In many cases during late April, May and June, when the testis is well filled with spermatogenic cells and the ducts are enlarged and filled with sperm, between these ducts cells can be found with a larger amount of cytoplasm than is present in other cells of the connective tissue. Some of these are pale yellow, others light pink in the sections, and some have vacuoles which may be empty or may contain a pink-staining homogeneous substance. Their position and appearance suggest that it is these cells which develop into the shrunken,

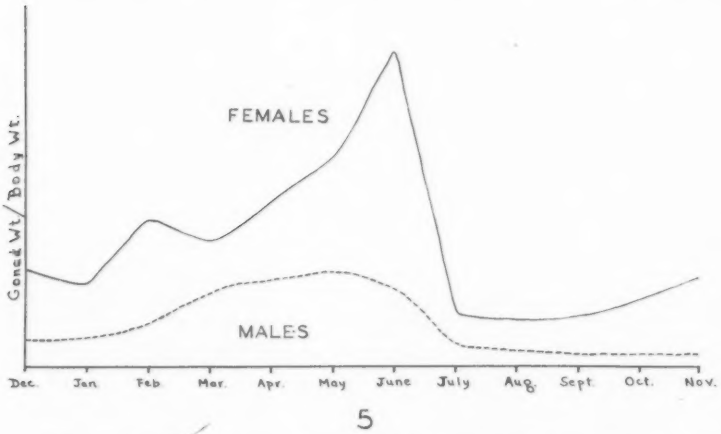

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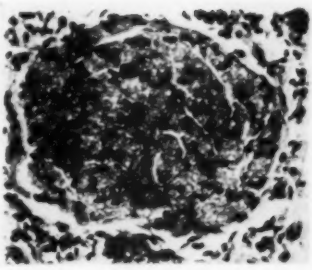
FIG. 5. Seasonal variation in gonad weight, expressed as percentage of body weight. Dotted line: testis; solid line: ovary.

vacuolated, pale yellow cells found in testes fixed after the sperm have been shed. They are the only cells found which might be called interstitial cells. There is no evidence, however, that they are secretory. Certainly the shrunken cells found after the sperm are shed resemble atrophic rather than active cells.

The ovary of *Fundulus* is unpaired but is bi-lobed at its anterior end and has a mid-ventral groove that separates it superficially into right and left portions. It occupies the same position in relation to other organs of the body cavity as does the testis in the male. It is covered externally with a layer of peritoneum beneath which is a thin tunica albuginea of connective tissue in which may be found smooth muscle



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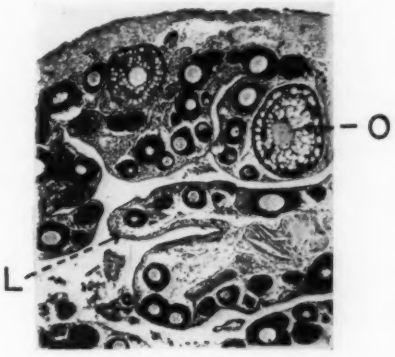

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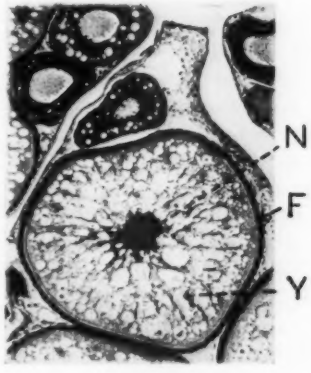

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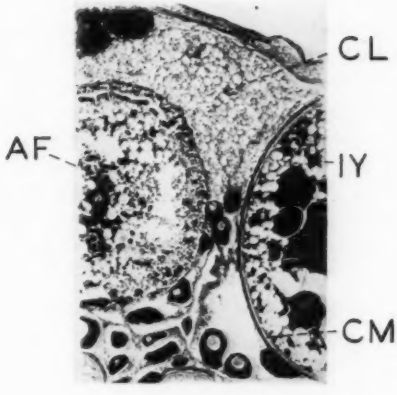

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PLATE II

FIG. 6. Interstitial cells of testis. × 340.

FIG. 7. Cross-section of ovary of animal killed 9/22/36. × 47. *O*, oöcyte showing vesicular yolk; *L*, ovigerous lamella.

FIG. 8. Oöcyte from animal killed 3/16/37. × 80. *F*, follicle; *N*, nucleus with several peripheral nucleoli; *Y*, vesicular yolk.

FIG. 9. Ovary of animal killed 6/11/36. × 47. *AF*, atretic follicle with secondary yolk present; *CM*, chorionic membrane of nearly ripe follicle; *CL*, "corpus luteum"; *IY*, secondary yolk.

fibers (Fig. 7). These smooth muscle fibers are most conspicuous in ovaries containing ripe or nearly ripe eggs or in those from which the eggs have been recently shed. Within the tunica albuginea are many ovigerous lamellæ which project into the lumen of the ovary. These folds are covered with an epithelial lining continuous with that of the oviduct, a single short structure leading from the lumen of the ovary to the genital opening posterior to the anus. The condition in the female differs from that in the male in that the external genital opening is found at the tip of a fold of skin which carries the opening a short distance posteriorly along the anterior ventral border of the anal fin.

Sections of the ovary made after the spawning period show that all mature oöcytes have either been shed or have become atretic. In these ovaries there can be found, just beneath the epithelium covering the ovigerous lamellæ, numbers of small cells with large, vesicular nuclei, each with a prominent nucleolus. Practically no cytoplasm is visible. While these cells may be found at any period of the year, the large number of mitotic figures found in them after the spawning season indicates that this is a period of active proliferation. In subsequent development both nucleus and cytoplasm enlarge, the latter the more rapidly, and the number of nucleoli increases from one to several. The cytoplasm is deeply basophilic. In later stages yolk vesicles appear (Fig. 7), few at first but increasing in number until the oöcytes are completely filled with these save for a peripheral ring of granular cytoplasm. By the time this condition has been reached the nucleus presents numerous nucleoli which are arranged in a ring just beneath the nuclear membrane (Fig. 8). The wall of the follicle, which at first consisted of only a few flattened cells, is now composed of a large number of cuboidal cells beneath which the chorionic membrane makes its appearance. In subsequent development there appears within the primary yolk vesicles what Guthrie (Marza, Marza, and Guthrie, 1937) calls intravesicular yolk (Fig. 9). While the contents of the primary vesicle are dissolved out in preparing the sections, this secondary yolk appears in sections as a homogeneous mass which stains faintly reddish with eosin. This intravesicular yolk increases in amount and at the same time yolk of the same sort appears between the vesicles and also increases in amount. The fully ripened oöcyte is completely filled with yolk except for the nucleus, located at one pole, and a few peripheral vacuoles. The chorionic membrane becomes thick and brittle. After the egg is discharged from the follicle the follicular epithelium undergoes marked hypertrophy, the cells developing large amounts of some substance which is dissolved out during preparation of the sections, leaving large vacuoles in the cells which

thus resemble fat (Fig. 9). Mitotic figures have not been found in these follicular cells during the formation of this structure. This "corpus luteum," which is actually yellow in color in the fresh condition and can be seen on the surface of the ovary as a yellow spot, gradually shrinks and disappears, though traces of it may be found as late as December or January.

During this process of maturation of the oöcytes some of the follicles undergo atresia. This is particularly noticeable when the spawning season approaches and the ovary is greatly distended by the growth of many follicles. The epithelium of the atretic follicle, provided atresia occurs late in development, may undergo changes similar to those described for the epithelium of the ruptured follicle, though not as marked in degree. The smaller size of the atretic follicles as well as the remains of the oöcytes within them serve to distinguish them from the corpora lutea developed in follicles from which eggs were shed (Fig. 9).

Certain seasonal changes in the ovary may be noted. At the end of the spawning season, late in July, all ripe eggs have been expelled and there is found in the ovary corpora lutea and atretic follicles as well as all stages in the development of oöcytes from the initial stage up to and including the late development of vesicular yolk. As has already been mentioned, the smallest recognizable stage of the developing oöcyte can be found just within the epithelium lining the ovigerous lamellæ at any time throughout the year, so it is conceivable that these begin maturation at different times during the year. Hence it is not clear whether these larger oöcytes found at the end of the spawning period belong to the same generation of cells as those which matured completely and were shed, or whether they are maturing oöcytes which began development somewhat later.

Changes in gross appearance and in weight of the ovary are more striking than similar changes in the testis. The "ripe" ovary is recognizable at a glance by the presence of numerous ripe eggs which appear as large, pale, semi-transparent spheres that cause the wall of the ovary to bulge at numerous points. The exhausted ovary, on the other hand, is very much reduced in size and the largest oöcytes which it contains are much smaller than ripe eggs and are yellowish and opaque. The weight changes are indicated in Fig. 5, comprising the data from 98 females. Sections of these ovaries show clearly the relation between the weight curve and the structure of the ovary. Just after the spawning period the ovary comprises the smallest percentage of the total body weight. From this point on there is a gradual increase from month to month in the weight of the ovary,

paralleled by an increase in size of the follicles and their contained oöcytes and in the number of yolk vesicles. The chorionic membrane appears as early as October in a few oöcytes, the number of chorionic membranes and the amount of yolk increasing during subsequent months. Droplets of intra-vesicular yolk may appear as early as March or even February, but rapid development of this secondary yolk takes place late in April, increasing rapidly in amount from then up to the time of spawning. The maximum percentage of the body weight formed by the ovary is found in June at the beginning of the spawning period. From this high point the weight of the ovary falls off rapidly as the eggs are shed to reach its lowest value in July at the end of spawning, then gradually increasing again to its maximum weight at the beginning of the next spawning period.

From this examination of the gonads of *Fundulus* collected at various times throughout the year it can be seen that the weight and the gross and microscopic appearance of both testis and ovary vary at different periods of the year. The differences are great enough to afford a definite index to the activity of the gonads. By comparing the weight and microscopic appearance of the gonads of experimental animals with this normal series the degree of sexual activity of such experimental animals and their position with reference to the normal sex cycle may be determined.

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THE CHEMICAL NATURE OF GROWTH FACTORS REQUIRED BY MOSQUITO LARVÆ

I. Riboflavin and Thiamin

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Previous work (1–4) has shown that the larvæ of the yellow fever mosquito (*Ædes ægypti*) require certain accessory growth factors which they normally obtain from living microörganisms. A proper quantity of living yeast suspension in distilled water supports good growth of the larvæ, but the same quantity of heat-killed yeast suspension under sterile conditions supports only a very slight growth. If, however, it is supplemented with an adequate amount of autoclaved liver extract, growth proceeds at a maximum rate. Liver extract without yeast gives no growth. Very large amounts of heat-killed yeast suspension (30 to 40 times as much as is required in the presence of liver extract) support fair growth even in the absence of liver extract. Thus at least two substances or groups of substances are required by the larvæ. Only one of these (designated as growth factor A) is present in liver extract. Both are present in heat-killed yeast, but much more yeast is needed to furnish adequate amounts of the one present in liver extract than of the other (4).

The sources and some of the properties of both growth factors have been described in previous publications (2, 4). This paper will show that riboflavin is one of the essential substances supplied by growth factor A and that thiamin (vitamin B_1) is an essential substance supplied by both of the crude growth factor preparations.

Methods

The Larval Growth Test

All the tests were conducted in 18 × 160 mm. test tubes holding 6 ml. of the medium to be tested. The various constituents of the medium were prepared as separate stock solutions adjusted to a pH of 5.8–6.0 and autoclaved ½ hour at 120° C. The required amounts

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of each solution were then pipetted into each experimental tube and made up with sterile distilled water to a total volume of 6 ml. When the growth factor A activity was being measured, each tube received 0.3 ml. of a washed, killed yeast suspension in distilled water (4).

In some experiments concerned with the factor A activity, and in all the experiments concerned with the other factor and with thiamin, the killed yeast was replaced by yeast extract (yeast vitamin Harris) and casein. Since the yeast extract used contains factor A (4) it was necessary first to treat it for ½ hour with 10 grams of fuller's earth to 100 ml. of 5 per cent solution of yeast vitamin Harris. The fuller's earth was then removed by centrifugation. This procedure removes much of the factor A (4). Amounts of treated yeast extract which supply enough of the second factor when A is supplied in some other form, do not contain enough factor A to support good growth in the absence of added A. In experiments in which whole yeast was replaced by yeast extract and casein, it was also necessary to add Ca^{++} (3), and this was provided as $CaCl_2$ at a concentration of 0.01 M.

Each experimental tube was inoculated with 3 newly-hatched bacteria-free *Ædes ægypti* larvæ secured by the methods previously described (4). Duplicate tubes were prepared for each medium in each experiment. The tubes were incubated at 28 ± 1° C. and were observed daily for the first 10 days and every other day thereafter, the number of larvæ in each stage of development being noted. From the data thus obtained, the rate of development could be expressed as a number, $N \times 1/T$, in which N is the percentage of larvæ reaching the 4th (last) larval instar within 10 days, and T is the average time in days required by these larvæ to reach the 4th instar. The adequacy of this growth index in the measurement of growth factor A has already been shown (4). Under optimum conditions, at 28° C., $N \times 1/T = 100 \times 1/4 = 25$.

It cannot be too strongly emphasized that significant results with the growth factors here concerned can be obtained only if the larvæ are reared in the complete absence of living microörganisms. To this end, the ordinary bacteriological methods to insure sterility were used and the sterility of each tube was tested by inoculation of a loopful of material to dextrose digest agar. With doubtful or particularly important tubes, broth and cooked meat under vaseline were also inoculated. Contaminated tubes, which occurred but rarely, were discarded.

Preparation of the Flavin-Purine Complex

The 60 per cent alcohol precipitate obtained in the preparation of Cohn's Fraction G (5) was extracted 3 times with 10 volumes of hot

75 per cent acetone. The filtrate was cooled and concentrated. When this was kept overnight in the cold room a heavy precipitate separated out.

This granular yellow precipitate, 20 mg. of which are derived from 100 grams of liver, has a nitrogen content of 28 to 30 per cent. Dr. Max Tishler found that it contains 80 to 85 per cent xanthine by the perchlorate precipitation of Biltz. Mr. Edward Meilman, working with one of us, found it to contain adenosine and sometimes inosine, glutathione, and traces of nicotinic acid amide. The precipitate regularly contains 1.2 per cent flavin phosphoric acid. Its further composition is being investigated.

Preparation of the "Alcohol-Ether Filtrate" and the "Calcium Filtrate"

The charcoal elute described by Subbarow, Jacobson and Fiske (6) was precipitated with 10 volumes of 95 per cent alcohol and 10 volumes of ether. The mixture was left in the cold room for 48 hours and the precipitate filtered off. The filtrate, after removal of the alcohol and ether, was the alcohol-ether filtrate fraction. To prepare the calcium-filtrate fraction, the filtrate was concentrated to a small volume and the concentrate neutralized with $Ca(OH)_2$ and precipitated with 5 volumes of alcohol. The calcium precipitate mostly contained glutathione and adenylic acid. The filtrate was concentrated to remove alcohol. So far, from the filtrate small amounts of ergothionine, tryptophane, tyrosine, nicotinic acid amide, guanosine and ethanol amine have been isolated.

Results

The Resolution of Growth Factor A into Two Fractions

Growth factor A was at first considered to be only one substance, but preliminary attempts at its purification soon indicated that the factor A activity was due to at least two substances. Treatment of a partly purified liver extract in 80 per cent alcohol with barium hydroxide gave a precipitate and a filtrate, each of which, after regeneration, had a very low activity. But combination of both fractions almost restored the original activity.

In a previous paper (2) it was shown that treatment of liver extract by charcoal removes the factor A activity. At that time no successful elution of the active material from the charcoal had been accomplished. Meanwhile, Subbarow, Jacobson and Fiske (6) had obtained a partially purified anti-pernicious anemia material prepared by adsorption on charcoal and subsequent elution with alcohol. This elute gave fairly good growth of the *Ædes ægypti* larvæ only if used at relatively high concentrations, on the basis of the weight of liver from which it was

derived. It was then found that if this elute were supplemented with the flavin-purine complex prepared from liver, high values for $N \times 1/T$ could be obtained with much lower concentrations of the elute, although the flavin-purine complex alone had no factor A activity whatever (Table I). In the presence of the high concentrations of elute needed to obtain $N \times 1/T$ values of about 20 in the absence of flavin-purine complex, metamorphosis to the adult stage required a longer time and was accomplished by far fewer insects than in the presence of both elute and flavin-purine complex. A concentration of each of the two components, such that 100 ml. of medium contained that amount of each derived from 50 grams of liver, gave $N \times 1/T$ values of 20 or more, while half this concentration gave values of about 10. These results show that the amount of elute plus flavin-purine complex derived from 50 grams of liver has a factor A activity equivalent to that amount of Lilly liver extract No. 343 derived from about 12.5 grams of liver (a 0.5 per cent solution).

TABLE I

The effect of the flavin complex

Concentration as grams of liver from which was derived amount of fraction present in 100 ml. of culture medium		
Charcoal elute	Flavin complex	$N \times 1/T$
600	0	18.2
400	0	20.0
200	0	13.3
100	0	2.1
200	200	23.7
100	100	25.0
50	50	20.0
27	27	10.1
13.5	13.5	1.7
6.7	6.7	0
0	50	0

The growth factor present in the charcoal elute has been subjected to further purification, the activity going into the alcohol-ether filtrate and the calcium-filtrate fractions prepared as already described. In the presence of suitable concentrations of these more highly purified fractions, the same amounts of flavin-purine complex gave the same growth as in the presence of the charcoal elute itself. The nature of the substances responsible for the activity of the calcium-filtrate fraction is now being investigated.

Comparison of the Effects of Riboflavin and the Flavin-Purine Complex

Repeated experiments have shown that, in the presence of the alcohol-ether filtrate or the calcium filtrate, pure crystalline riboflavin will support excellent growth of the larvæ (Table II). Very much

TABLE II

Comparison of the effects of flavin complex and riboflavin. Each of the tubes contained for lines 1–12, alcohol-ether filtrate, for lines 13–24, calcium filtrate, each at a concentration such that 100 ml. of solution contained the material derived from 53 grams of liver.

Concentration mg. per 100 ml.		$N \times 1/T$	Percentage of larvæ transforming to adults	Percentage of larvæ transforming to normal adults
Flavin complex	Riboflavin			
0	0	0	0	0
20.0	0	23.1	100	100
10.0	0	23.1	67	67
5.4	0	6.5	33	0
0	1.0	16.6	50	0
0	0.5	15.8	33	0
0	0.33	10.7	0	0
0	0.17	10.7	17	0
0	0.08	13.4	33	0
0	0.04	14.1	33	0
0	0.025	14.1	33	17
0	0.008	6.0	33	17
0	0	0	0	0
13.4	0	24.0	100	100
10.0	0	18.9	67	67
6.6	0	23.1	100	100
3.3	0	14.8	50	50
0	0.25	19.8	67	0
0	0.17	21.4	83	0
0	0.125	18.0	67	0
0	0.080	19.8	67	50
0	0.040	22.2	67	33
0	0.017	14.8	67	33
0	0.008	6.2	17	17

lower concentrations of riboflavin are required than of the flavin-purine complex. In the presence of alcohol-ether filtrate, as little as 0.025 mg. of riboflavin per 100 ml. gave growth almost as good as did 1 mg. of riboflavin, but somewhat less than that obtained with 10 mg. of flavin-purine complex; 0.008 mg. of riboflavin per 100 ml. gave growth as good as 5.4 mg. of flavin-purine complex. In the presence of the calcium filtrate, 0.04 mg. of riboflavin per 100 ml. gave a value for $N \times 1/T$ as high as that obtained with 6.6 mg. of flavin-purine

complex, while 0.017 mg. of riboflavin was about equivalent to 3.3 mg. of flavin complex. Since the flavin complex contains 1.2 per cent flavin-phosphate, it is readily apparent that the flavin as contained in the complex has about the same activity, so far as larval growth is concerned, as free riboflavin. But it was soon noted that many of the larvæ growing in media containing riboflavin in place of flavin complex had difficulty in metamorphosing into the adult stage. This fact is well shown in Table II. In the presence of alcohol-ether filtrate, those concentrations of flavin complex which gave the highest $N \times 1/T$ values permitted 67 to 100 per cent of the larvæ to emerge as adults and all the adults which emerged were normal and vigorous. Adults were considered normal if they were able to emerge completely from the pupal sheath and to fly up from the surface of the liquid. Abnormal adults were those which either failed to emerge completely from the pupal sheath, or, having done so, were too weak to get free from the surface of the liquid. In Table II it may be seen that in media containing alcohol-ether filtrate and concentrations of riboflavin giving high $N \times 1/T$ values, very few adults emerged and most of these were abnormal. Larvæ which failed to transform into adults usually died, either in the late larval stage or in the pupal stage. In the presence of calcium filtrate, the same differences are apparent between flavin complex and riboflavin. Here again, riboflavin in the most favorable case permitted only 50 per cent of the larvæ to transform into normal adults. With flavin complex, concentrations which gave the fastest larval growth also gave the highest percentage of normal adults, but with riboflavin the highest percentage of normal adults was in general obtained with concentrations which gave submaximal larval growth rates.

Riboflavin is thus an essential substance for the growth of mosquito larvæ. Flavin-purine complex, however, contains some other substance which is possibly of little or no importance for larval growth but is very important for metamorphosis. The nature of this material is unknown. Perhaps the difference between the effects of the flavin complex and the riboflavin may be accounted for by the fact that the flavin in the complex exists as flavin phosphate.

The Need for Thiamin

It has already been shown (2) that if larvæ of *Ædes ægypti* are grown with casein, $CaCl_2$, liver extract, and yeast extract previously treated with fuller's earth, both the factors present in liver extract and those present in the yeast extract withstand 5 hours of autoclaving at 120° C., if each extract is tested in the presence of the other extract

autoclaved for only ½ hour. But if both the extracts are autoclaved for 5 hours, the amount of growth obtained is greatly reduced (Table III). When such autoclaved liver and yeast extracts are supplemented with crystalline thiamin in low concentration, growth is restored almost to that obtained with extracts autoclaved for only ½ hour (Table III). This is true whether the casein be free from water-soluble vitamins only, or from both fat- and water-soluble vitamins, although with the latter type of casein, growth is never as good as with the former, possibly because of a deficiency in a fat-soluble growth factor. Table III also shows the interesting fact that a thiamin solution autoclaved for 5 hours is fully as effective as one sterilized by Berkefeld filtration. This can only mean that certain decomposition products of thiamin are as effective as the intact compound. These decompo-

TABLE III

The effect of thiamin. Each tube contained 0.01 M $CaCl_2$.

Type of casein	Treatment of liver extract (0.5% Lilly 343) and yeast vitamin Harris 0.33% after fuller's earth adsorption	Concentration of added thiamin mg./100 ml.	$N \times 1/T$ Exp. A	$N \times 1/T$ Exp. B
Harris. Free from water-soluble vitamins	Autoclaved ½ hour	0	23.1	25.0
" " " " "	Autoclaved 5 hours	0	6.2	7.5
" " " " "	" " "	0.80	20.7	16.7
" " " " "	" " "	0.33	17.6	15.4
" " " " "	" " "	0.17	15.4	16.7
" " " " "	" " "	0.08	—	18.2
" " " " "	" " "	0.33 —autoclaved 5 hours	18.8	20.0
Harris. Free from water- and fat-soluble vitamins	Autoclaved ½ hour	0	18.8	14.3
" " " " " " "	Autoclaved 5 hours	0	0	0
" " " " " " "	" " "	0.80	12.8	9.6

sition products apparently are not formed when liver extract or yeast extract is autoclaved for 5 hours. Presumably, under these conditions, other less easily utilized products are formed. This accords with the fact that growth does go on even to the adult stage in the medium autoclaved for 5 hours and not supplemented with thiamin. The slower rate and greater difficulty with which it proceeds are perhaps the result of a slow synthesis of an essential substance which is readily synthesized from thiamin itself or certain of its decomposition products. The utilization of certain decomposition products of thiamin has been shown for *Phycomyces* by Schopfer and Jung (7), for excised tomato roots by Robbins and Kavanagh (8) and for *Staphylococcus aureus* by Knight (9).

DISCUSSION

Early in the study of the nutritional requirements of insects, it became apparent that yeast and other microörganisms were the

natural source of certain necessary foodstuffs (10–14). Several workers, as for example Bacot and Harden (15), working with *Drosophila*, and Richardson (16) with *Ephestia kuehniella*, soon found that yeast extract would supply substances essential for the growth of insect larvæ. Since yeast extract was at that time synonymous with vitamin B, these authors concluded that insects require vitamin B. Similar evidence for the vitamin B requirement of insects has been recently supplied by McCay (17) for the roach *Blatella germanica*, by Crowell and McCay (18) for larvæ of the clothes moth *Tineola bisseliella* and by Frost, Herms and Hoskins (19) for larvæ of the mosquito *Theobaldia incidens*. Hobson (20), and Street and Palmer (21) were among the first to show that the yeast extract supplied several different essential substances, and it was Hobson who found that pure vitamin B_1 is one of these substances. Van't Hoog (22) has gone still further and shown that both crystalline riboflavin and thiamin are substances essential for the growth of larvæ of *Drosophila melanogaster*. Growth does not occur, however, unless still a third substance or group of substances, also present in yeast extract, is supplied. Thus the water-soluble accessory growth factors required by *Drosophila melanogaster* and *Ædes ægypti* are identical so far as they are known at present. Both insects require for larval development riboflavin, thiamin, and a third factor present in yeast extract, liver extract, rice-polishings concentrate, etc. It is interesting that these requirements form a perfect parallel to the vitamin B complex requirements of vertebrates (23–25).

The nature of the third factor required by vertebrates and present in liver extract is now under investigation in several laboratories. The indications are that nicotinic acid amide or cozymase is one of the essential substances present in the third factor, but not the only one (26–29). Lepkovsky (30) has recently isolated one of these substances in crystalline form, but he gives no information as to its nature or method of preparation. So far as *Ædes ægypti* larvæ are concerned, nicotinic acid or its amide cannot replace the "calcium-filtrate" fraction of liver extract. But evidence is now accumulating that nicotinic acid amide plus certain other substances can at least partially replace the impure liver fraction.

It is worth noting that while rice-polishings concentrates have been generally considered to be free from riboflavin (as in the work of Cook, Clarke and Light (28)), they must actually contain appreciable amounts, since a 0.4 per cent solution of Ryzamin B has been found (4) to give growth about as good as a 0.3 per cent solution of Lilly liver extract No. 343. On the basis of this result, a 0.4 per cent

solution of Ryzamin B should contain around 0.02 mg. of riboflavin per 100 ml. It is not surprising that the clay adsorbate (the International Standard vitamin B_1) prepared from rice-polishings concentrate also can replace liver extract (4), since the flavin and other necessary factors would be adsorbed as readily as the thiamin.

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Summary

Riboflavin, and thiamin or certain of its degradation products, are necessary for the normal development of larvæ of the yellow fever mosquito *Ædes ægypti*. In the presence of all the other necessary growth factors, riboflavin permits normal larval growth at concentrations as low as 0.0004 mg. per ml. and has a definite effect at a concentration of 0.00008 mg. per ml., while thiamin permits normal growth at concentrations at least as low as 0.0008 mg. per ml. Although larval growth is normal in the presence of suitable amounts of heat-killed yeast, riboflavin, and the other liver extract factors supplied in partially purified form, metamorphosis to the adult stage is successfully accomplished by a relatively small percentage of the insects. If, however, the flavin is supplied as a flavin-purine complex containing 1.2 per cent flavin-phosphate, almost all the larvæ transform into vigorous adults. The nature of the additional factor responsible for this difference is not known.

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COPULATION IN THE ACŒLOUS TURBELLARIAN POLYCHŒRUS CARMELENSIS[1]

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Attention has been directed recently to the primitive condition of the reproductive system of the Turbellaria Acœla, as compared with most of the Platyhelminthes, and to the occurrence of copulation in the group (Hyman, 1937). The Acœla are hermaphroditic. Most members of the group possess a copulatory mechanism including an extensible penis and a seminal receptacle, provided with one or more small tubes called "Mundstücke," the function of which is assumed to be the transport of sperm from the receptacle or bursa toward the eggs. In view of the presence of a copulatory mechanism, von Graff (1905) assumed that true copulation is of widespread occurrence in the group.

Gardiner (1898) had earlier concluded that in *Polychœrus caudatus* sperm transfer is accomplished by a process of hypodermic impregnation, in which the "mouth pieces" function as hypodermic organs. Bresslau (1933) and Hyman (1937) admit that hypodermic impregnation may be of common occurrence in the Acœla. We have found no specific account of this process other than that of Gardiner (1898). Only two descriptions of true copulation in the Acœla have come to our attention, that of Peebles (1915) for *Monochœrus lineatus* and that of Hyman (1937) for *Amphiscolops langerhansi*. Only in the latter case was the observation of living animals supplemented by the study of sectioned pairs. The study of both living and sectioned specimens of *Polychœrus carmelensis*, a form very closely similar to *P. caudatus*, has yielded no evidence of the occurrence of hypodermic impregnation. The evidence demonstrates conclusively that impregnation is regularly accomplished by means of true copulation, the details of the process differing considerably from that described by Hyman for *Amphiscolops langerhansi*.

During the summer months of 1936 and 1937 *Polychœrus carmelensis* were collected in great abundance from tide pools at Pescadero Point on Carmel Bay, California, and in smaller numbers from tide pools elsewhere in the vicinity. Examination of living and sectioned specimens soon revealed certain structural differences from the Atlantic

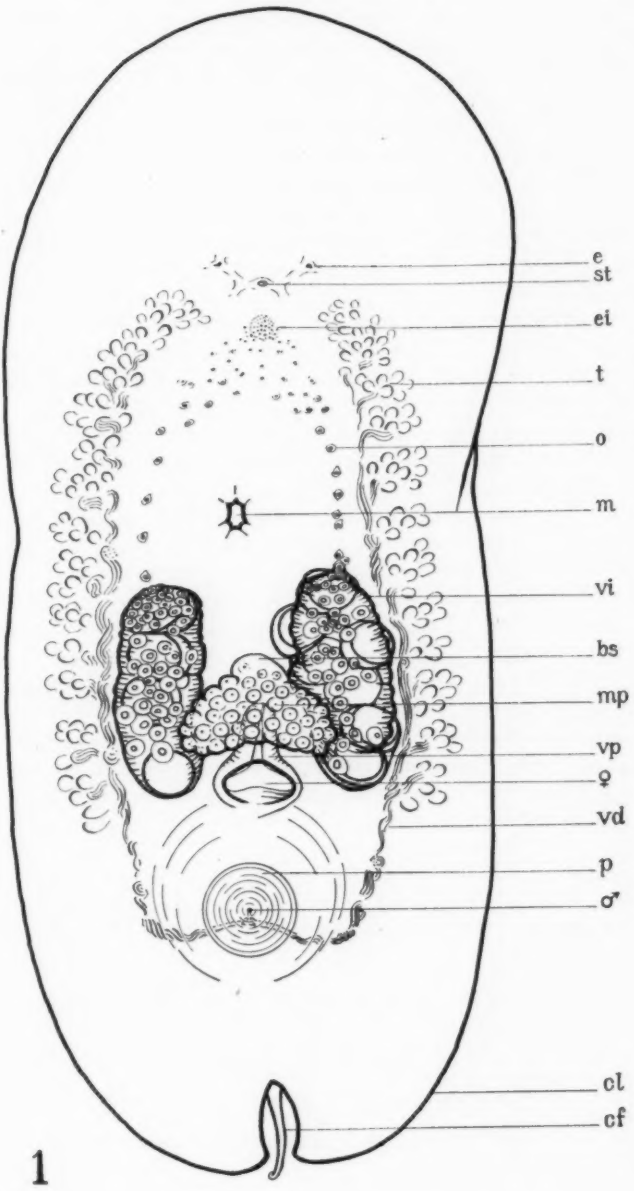
[1] The authors are indebted to the Society of Sigma Xi for aid in the publication of this work.

Coast *Polychærus caudatus*, as described in the papers of Mark (1892), Verrill (1893), Gardiner (1895, 1898), and Löhner (1910). On the basis of these differences the Pacific Coast form was designated as a distinct species (1938).

A brief description of the reproductive system of *P. carmelensis* will be presented in order to facilitate an understanding of the copulatory process. Previously, the reproductive system has been discussed only with respect to certain points in which it differs from that of *P. caudatus* (1938).

The reproductive structures of *P. carmelensis*, in ventral view, are illustrated diagrammatically in text fig. 1. The female system includes a pair of gonads which appear to originate anteriorly in a median "Eilager" (*ei*), a short distance posterior to the statocyst (*st*). Extending posteriorly from the Eilager are two lateral ventral rows of oöcytes (*o*) which, a short distance below the level of the mouth, become embedded in the vitellaria (*vi*), the latter terminating at the level of the female aperture (♀). In the vitellaria the young oöcytes are located ventrally, and move dorsally as they mature. The female gonads are typical of the Acœla in their lack of specialized investing walls and of ducts to either the exterior or to the bursa seminalis. The external female aperture is roughly elliptical, about one-sixth the width of the body, enormously distensible. From this aperture there extends dorsally, to a distance of about two-thirds the thickness of the body in this region, a large dorso-ventrally flattened chamber, the vagina. The anterior wall of the vagina bears, at a position somewhat dorsal to its mid-point, a thick-walled pocket (*vp*) which is directed anteriorly, slanting slightly dorsally, and provided with a small lumen. The anterior end of the pocket is embedded in the dorsal part of the bursa seminalis (*bs*), its lumen opening directly into the bursa much in the manner in which Mark described the vagina of *P. caudatus* as communicating at its dorsal end with the naked bursa tissue. We find in the literature no mention of a structure in *P. caudatus* corresponding to the vaginal pocket of *P. carmelensis*. The muscular walls of the vagina and pocket are lined with a nucleated epithelium lacking cilia. These structures are shown, in sagittal section, in plate figures 1 and 2.

TEXT FIG. 1. Ventral view, showing arrangement of male and female reproductive systems. Composite drawing, with projector, from two whole mounts stained with Delafield's hæmatoxylin, the testis follicles largely diagrammatic. Magnification 35 ×. *bs*, bursa seminalis; *cf*, caudal filament; *cl*, caudal lobe; *e*, eye; *ei*, "Eilager"; ♀, external female aperture; ♂, external male aperture; *m*, mouth; *mp*, mouth piece of bursa; *o*, oöcyte on way from Eilager to vitellarium; *p*, penis bulb; *st*, statocyst; *t*, testis follicles; *vi*, vitellarium containing young oöcytes and mature eggs; *vd*, vas deferens; *vp*, vaginal pocket.



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The bursa seminalis appears to resemble that of *P. caudatus* in all essential features. It is somewhat kidney-shaped, and is located medially between the two vitellaria, anterior to the female aperture and the vagina. It occupies the space between the vaginal pocket and the ventral body wall but extends anteriorly well beyond the pocket, the anterior dorsal portion surrounding the anterior tip of the pocket. The internal structure of the bursa is shown, in slightly oblique transverse section, in plate fig. 3. The entire ventral part of the bursa is composed of many small compartments, each containing a small tubular "mouth piece" (*mp*), the long axis of which approximately parallels the dorso-ventral axis of the body. The ventral small ends of these tubes open into the vacuolated marginal parenchyma (*va*), a thin layer of which separates the bursa from the ventral integument. At the basal end of each mouth piece is usually attached a mass of sperm (*s''*) occupying a small vacuole. The ventral compartments of the bursa merge dorsally into a chamber (*bs'*) which frequently contains scattered sperm masses sometimes connected with the ventral masses.

Regarding the histology of the bursa a few facts may be mentioned. The syncytial tissue of which the bursa is composed sometimes appears almost continuous (plate fig. 3) except for the presence of sperm masses, muscle fibers and a few small vacuoles. The highly vacuolated appearance, especially of the dorsal part of the bursa, which seems to characterize many specimens (plate fig. 2), probably results largely from the disruptive influences of mating and egg-deposition, although the method of fixation is to some extent responsible, slowly penetrating fixatives permitting time for violent muscular contraction and consequent distortion of tissue. The bursa lacks a differentiated limiting wall. Ventrally, it impinges upon marginal parenchyma (in the terminology of Löhner); dorsally, it is bounded by the anterior pocket posteriorly and central parenchyma anteriorly (plate figs. 1 and 2); its anterior curved surface approximates the intestinal parenchyma (*ip*) from which it is separated by muscle fibers; laterally, it is enclosed by the vitellaria. The bursa communicates with the exterior by way

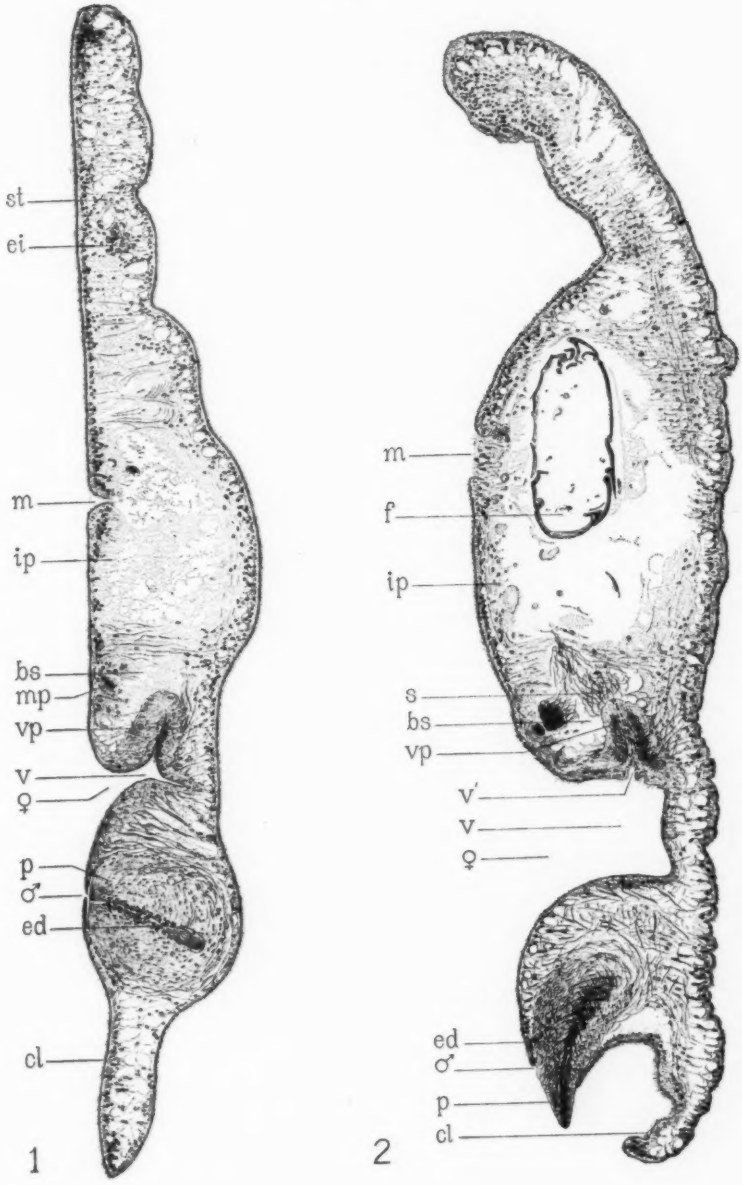
PLATE I

Median sagittal sections of a non-copulant individual (Fig. 1) and a copulant individual (Fig. 2), for comparison of the genital regions. Drawn with aid of projector. *ed*, ejaculatory duct; *f*, food organism; *ip*, intestinal parenchyma; *s*, newly-acquired sperm; *v*, vagina; *v'*, opening of lumen of vaginal pocket into vagina; other abbreviations as used in text fig. 1.

FIG. 1. Fixed in Heath's solution, stained with Heidenhain's hæmatoxylin. Magnification 51 ×.

FIG. 2. Fixed in Champy's fluid, stained with Heidenhain's hæmatoxylin. Magnification 47 ×.

PLATE I



of the small lumen of the vaginal pocket, the vagina and the female aperture.

The male reproductive system (text fig. 1) includes a pair of gonads of follicular arrangement (*t*), extending from approximately the level of the statocyst to a point somewhat posterior to the female aperture. They are lateral and dorsal to the female gonads. The vasa deferentia (*vd*) are a pair of channels in the parenchyma through which sperm pass from the follicles of each side to the ejaculatory duct (plate figs. 1 and 2, *ed*) of the penis (*p*). The testes and vasa deferentia lack specialized investing walls; the ejaculatory duct is lined with a nucleated epithelium. The penis is located posterior to the vagina. It is compact, muscular; in the retracted state, an ovoid bulb, which occupies almost the entire thickness, and nearly one-fourth of the width, of the body in this region (plate fig. 1, *p*). Its dorsal end is inclined somewhat posteriorly. In the retracted state the ventral tip of the penis extends free into a small circular chamber which is covered by ventral integument bearing a very small, but greatly distensible, perforation, the male aperture (♂). During activity the penis is protruded through the male pore, and assumes an elongated tapering conical form, arched so that the distal end is directed posteriorly (plate fig. 2, *p*).

The copulatory mechanism of *P. carmelensis* consists, in brief, of the male organ and aperture, the female aperture, the vagina with anterior pocket, and the bursa or seminal receptacle.

Material and Procedure

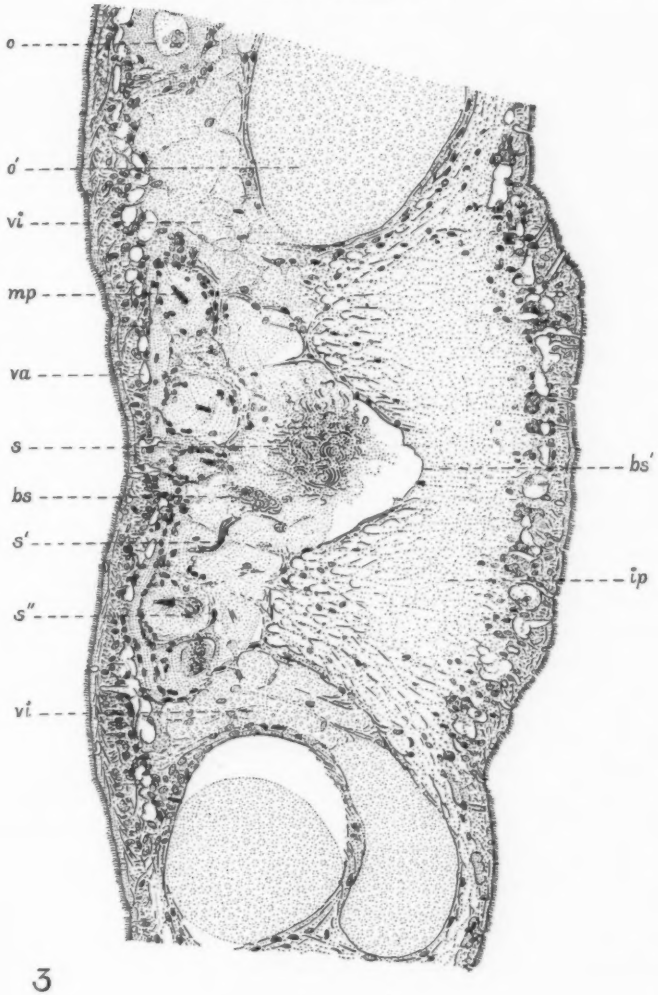
Collections of *Polychærus carmelensis* were brought into the laboratory on the *Ulva* (and a few other sea-weeds) upon which they are found in the tide pools. Large numbers of individuals of all ages were removed immediately from the *Ulva* and preserved for later study. Usually a few hundred worms were distributed among several finger-bowls. Most of the remainder of the *Ulva*, bearing thousands of worms and abundant food organisms (chiefly small crustacea), was placed into two running salt-water aquaria. Here the worms remained alive and active for long periods of time.

During the summer of 1936 our attention was focused mostly on

Plate II, Figure 3

Detailed portion of section of text fig. 3 (*a–b*), to show newly-acquired sperm mass (*s*) in the dorsal part of the bursa anterior to the vaginal pocket. Drawn with aid of projector. Magnification 180 ×. *bs′*, surface of dorsal chamber of bursa; *o*, young oöcyte, and *o′*, nearly mature egg, in vitellarium; *s′*, sperm, apparently migrating from mass (*s*) toward ventral bursa mouth piece; *s″*, sperm ball attached to mouth piece; *vα*, vacuole of ventral marginal parenchyma; other abbreviations as previously used.

PLATE II, FIGURE 3



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a study of the anatomy and development of *P. carmelensis*. All observations of mating activities were made in 1937, in the period between June 24 and August 24. Observations were rendered somewhat difficult by the extremely brief duration of mating; the entire process rarely exceeded a minute and a half. In all, 35 matings were witnessed; most of these were observed in detail, usually with the aid of a hand lens. Occasional pairs were removed to glass slides for immediate examination, and fifteen pairs were fixed for later sectioning and study. The pairs were transferred by means of a pipette from aquarium or fingerbowl to the fixative. Unfortunately, the mates separated in every case, either during this transfer or at some time before the embedding process was reached. This separation removed the possibility of subsequent identification of members of a single pair, since two or more pairs were usually stored in a single vial. Only two pairs were stored separately. However, the data furnish ample evidence with regard to the essential features of the mating process in *Polychœrus carmelensis*.

Observations

Copulation was most frequently observed on the glass sides of the aquaria where the worms were present in great numbers. About one-third of the observed matings occurred in the fingerbowls. Mating was never seen in the tide pools, but is assumed to have preceded the deposition of eggs which were found in abundance on small stones and in the folds of *Ulva*. In *Polychœrus carmelensis* the eggs are fertilized before deposition, and there is no evidence of the occurrence, in this species, of self-fertilization or of any method of sperm transfer other than true copulation.

The 35 matings witnessed in the laboratory are listed in Table I, according to the respective dates and hours of occurrence. The table shows that matings are infrequent during the late morning, afternoon, and evening, but are relatively numerous immediately after dawn, from 5 to 7 o'clock. Periods of observation of the aquaria and fingerbowls included all hours of the day and night except from 1 to 4 A.M. However, in the table, hours of observation (+) are indicated, where records are sufficiently complete, only for days upon which one or more matings was witnessed. While observations during the period from 4:45 to 9:30 A.M. were made only on the three dates listed, the observations during the usual working hours of the day, as well as at night, were far more extensive. Therefore, the difference in frequency of mating in the early morning as compared with the later hours is probably greater than the total numbers indicate. Indeed, after having repeatedly watched in vain for signs of mating, the occurrence

of nine or ten matings within 2 to 2½ hours after dawn seemed a veritable epidemic. It is interesting to note that, whereas early morning is the preferred mating time, egg-deposition occurs usually after sundown.

There was considerable variation in the degree of activity of the worms during the month of observation, probably in some measure correlated with the length of time during which they had been kept in the laboratory. However, our data are of insufficient extent to provide evidence of this correlation.

The process of mating took place in the following manner (text fig. 2). Considerable restlessness was evidenced by certain mature-looking worms, which usually contained visible accumulations of sperm in the vasa deferentia and at the base of the ejaculatory duct.

TABLE I

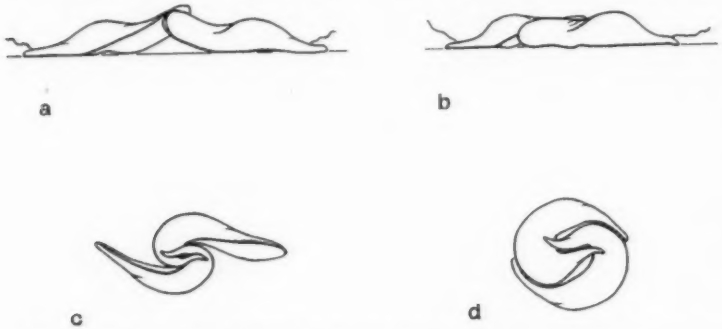
The numbers of matings observed in the laboratory are listed according to the dates and hours of occurrence. The hours of observation (+) on the respective dates are indicated, where the records are sufficiently complete.

	4:45–5 A.M.	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–1 P.M.	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	Total observed matings
June 24											1							1
July 28						3												3
29								1										1
31	+	5	4	+	+													9
Aug. 2						+	+	+				2					1	3
9	+	4	3	3		3					+	+	1			1	+	15
13							+	+	+	1	+							1
16						+	+	+	+	+	1	+						1
24		1	+	+						+	+	+	+					1
Total observed matings		10	7	3		6		1		1	2	2	1			1	1	35

Such individuals moved about actively, frequently elevating the head end, and pausing to exchange head contacts with other worms. In case such overtures between two worms were prolonged, the individuals faced each other with the anterior halves of their bodies touching ventrally and swaying slowly from side to side in opposite directions (*2a*). This process was sometimes terminated by one worm moving away. If continued, one animal presently bent its anterior half down under its partner, twisting at the same time so as to bring its ventral surface into contact with the ventral surface of its partner, and retaining attachment to the substratum along the posterior margin of one side. The partner at the same time arched dorsally, elevated the side adjoining the other individual, remaining attached only along the margin of one side (*2b*). The two worms then glided along each other in opposite directions, ventro-lateral margins in contact, until each

animal had passed more than half its length beyond its partner. At this point, when the copulatory organs were presumably in juxtaposition, one animal, with a sudden snap, released its attachment completely, both curled ventrally, the pair forming a ball attached only by the lateral marginal papillæ of one animal (2*c* and 2*d*). After a connection of only 40 to 50 seconds, the attached animal uncurled, and began to move off, necessitating the reattachment of its partner. The entire mating process, including overtures, occupied only 1 to 1½ minutes.

The mating position obviously conceals the actual relation of the genital regions of the two worms. Mating pairs placed on slides for examination separated immediately. However, they showed certain facts clearly. The genital region invariably evidenced activity by

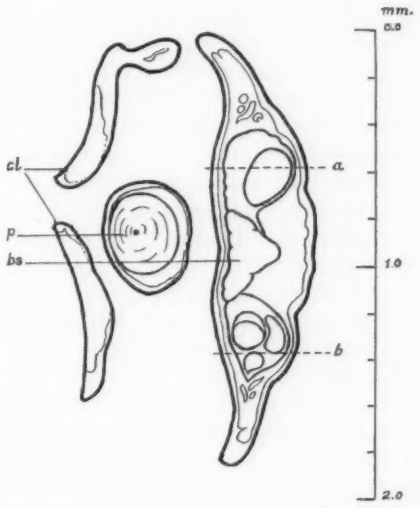


TEXT FIG. 2. Series of sketches showing progressive stages in the assumption of the typical copulatory position. *a*, *b*, mating pair viewed in plane parallel to the substratum; *c*, *d*, viewed in plane perpendicular to the substratum.

the protrusion of the penis, the repeated contraction and distention of the vagina and the female aperture. In several individuals a large mass of sperm was visible in the dorsal part of the bursa anterior to the vaginal pocket. Only once were sperm observed at the female aperture; there was never a trace of sperm in the vagina, pocket, or about the penis, though a fluid filled with oil-like globules issued from the penis of one individual. The presence of sperm in the bursa, and their complete absence from vagina, pocket or penis, suggested that sperm might be deposited directly into the dorsal chamber of the bursa by the insertion of the penis of one worm into the lumen of the vaginal pocket of its mate. Confirmatory and supplementary evidence was provided by the sectioned material.

A sagittal view of a typical individual fixed during copulation (plate fig. 2) shows striking differences from the typical non-copulant

(plate fig. 1). The body is usually curved ventrally though seldom retaining in fixation the full flexure of the mating position. Invariably, the penis (*p*) is protruded through the male aperture (♂), and the vagina (*v*) distended. The vaginal pocket (*vp*) is usually curved slightly ventrally, the epithelial lining of the lumen showing signs of activity in the antero-posterior orientation of the nuclei and the pseudopodial projections and occasional sloughing at the opening into the vagina. In the non-copulant the penis is retracted, the vagina and female aperture antero-posteriorly flattened. The pocket infre-



TEXT FIG. 3. Transverse section (slightly oblique dorso-ventrally) of individual fixed, during copulation, in Heath's solution and stained by the Flemming tri-color method. The ventral curvature typical of the copulatory position is shown by the presence of caudal lobes (*cl*), and penis (*p*), in section which passes through the anterior portion of the bursa (*bs*). Camera lucida drawing, with scale.

quently displays evidence of activity, and in some cases the lumen is not visible at the anterior end. A few individuals, such as that shown in text fig. 3, in transverse section, retained more completely the curvature of the typical copulatory position (text fig. 2, *c* and *d*). The body is bent ventrally at the level of the vagina, so that the transverse section through the anterior portion of the bursa (*bs*) passes also through the extended penis (*p*), and through the caudal lobes (*cl*). Thus, the copulatory organs of the fixed mating worms indicate activity and are in such a position as to permit the insertion of the

penis of each individual into the anteriorly-directed pocket of its mate.

The accomplishment of impregnation during copulation is evidenced by 16 of the sectioned worms. In these animals (plate figs. 2 and 3) a large mass of sperm (*s*) is found in the dorsal part of the bursa usually anterior to the pocket, but frequently connected by strands of sperm with the anterior end of the lumen. The absence of sperm elsewhere in the lumen, and in the vagina, in both fixed and living copulants, seems to justify the conclusion that the sperm are deposited directly into the dorsal chamber of the bursa by the insertion of the penis of the mate into the vaginal pocket, probably as far as the opening of the latter into the bursa. The large aggregates of sperm in the dorsal bursa are sometimes densely coiled (plate fig. 3), sometimes more scattered (plate fig. 2). They are embedded in the bursa tissue; several cases in which they appear to be embedded in the intestinal parenchyma probably represent distortion due to slow fixation. The enormous size of these sperm masses, their position anterior to the pocket, and their frequent connection with the pocket lumen, distinguish them from those found in random specimens. They are without doubt masses deposited during the mating which was terminated immediately before, or interrupted by, fixation. Three additional sectioned worms contained smaller masses of sperm which were judged to be newly-acquired. The remaining eleven worms gave no evidence of impregnation.

It is obvious that some of the fifteen pairs of worms were reciprocally impregnated since more than half of the individuals contained newly-acquired sperm. The two mating pairs which were preserved in separate vials were both cases of reciprocal impregnation. By noting for each vial in which more than one mating pair were preserved, the proportion of individuals containing newly-acquired sperm, it was found that at least six of the total fifteen matings involved reciprocal impregnation. Allowing for the disturbance of the mating worms incident to their transfer to the fixative, it appears probable that reciprocal impregnation is of more usual occurrence than the minimum figures indicate.

Thus, the results of examination of the fixed material are in harmony with, and supplement, the observations of the living mating worms. There was no evidence of the use of bursa mouth pieces for the accomplishment of sperm transfer by the hypodermic method, as postulated by Gardiner for *P. caudatus*.

The subsequent path of the sperm from the dorsal part of the bursa to the eggs will be considered only briefly at present. Undoubtedly,

some of the sperm migrate ventrally within the bursa to the mouth pieces, as evidenced by certain of the fixed copulants (plate fig. 3, *s'*). This is in accord with Mark's view of the distribution of sperm in the bursa of *P. caudatus*. We have as yet found no evidence of the passage of sperm through the mouth pieces to the eggs by way of the vacuolated ventral parenchyma, as Löhner postulated for *P. caudatus*. We hope to consider this subject more fully in a paper dealing with the maturation and fertilization of the eggs of *Polychœrus carmelensis*.

Discussion

Attention may be directed to certain similarities and differences in the copulatory process of the three species of Acœla for which it has been described, *Monochœrus lineatus*, *Amphiscolops langerhansi*, and *Polychœrus carmelensis*. The general copulatory position is similar in all cases in that the mates are united by the ventral surfaces of the posterior ends, their anterior ends extending in opposite directions. No further details of the process were given by Peebles (1915) for *Monochœrus lineatus*. In *Amphiscolops langerhansi* (Hyman, 1937), the mates assume the form of a ball for about thirty seconds prior to the assumption of the final mating position, in which they are unrolled and attached by their ventral surfaces. The ball form is closely similar to the final mating position characteristic of *P. carmelensis*. The prolonged union of forty to fifty-five minutes in *Amphiscolops* contrasts strikingly with the extremely brief union of forty to fifty seconds in *P. carmelensis*.

The relative positions of the reproductive organs of the mates differ, in the two species, in correspondence with structural differences. In *Amphiscolops*, the bursa opens into the female genital pore by way of a small invagination (Hyman, p. 322). The penis papilla is free only posteriorly, behind which there is a projecting sperm guide. Copulation occurs by the interlocking of penes. The sperm are directed toward the bursa of each individual by the sperm guide of the mate. Impregnation is mutual in *Amphiscolops langerhansi*.

Summary

The process of mating is described for *Polychœrus carmelensis*. A study of living and fixed mating individuals, separated from their mates, indicates that copulation involves the insertion of the penis of each individual into the vaginal pocket of the mate, so that sperm are deposited directly into the dorsal chamber of the seminal bursa. Impregnation is, in some cases, reciprocal. The entire process lasts only one to one and a half minutes.

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TRANSPLANTATION OF MUTANT AND WILD TYPE BRISTLE-BEARING TISSUES IN DROSOPHILA MELANOGASTER [1]

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INTRODUCTION

The data on mosaics (Sturtevant, 1932) show that certain bristle mutants such as scute and yellow, though ordinarily autonomous, may display non-autonomous development if present in small patches of tissue. Development of such characters as forked, singed and spineless, however, is not influenced by surrounding tissues of a different genotype (Morgan, Bridges and Sturtevant, 1919; Patterson, 1931).

Imaginal disks from which arise the bristle-bearing regions of adult flies are, on transplantation to host larvæ, known to reach a maximum degree of development. Their condition indicates that they may serve as a basis for the study of genic interaction in the same manner as have imaginal eye tissues (Ephrussi and Beadle, 1937; Beadle and Ephrussi, 1936).

Since the effects produced by imaginal eye disk transfer have led to the postulation of definite diffusible substances active in the production of eye color, it is reasonable to assume that the presence of similar substances, essential for the development of various bristle types, may be demonstrated. Plunkett (1926) has postulated that there may be something which, spreading from a region of maximum effect, tends (*a*) to remove bristles or (*b*) to form bristles. Additional evidence of the existence of such a substance is offered by Child (1935).

MATERIALS AND METHOD

The technique used in making transplantations in *Drosophila* has been so fully described (Ephrussi and Beadle, 1936) that a detailed repetition is unnecessary. An imaginal disk, dissected out of one larva, the donor, was picked up in a micro-pipette and injected into the body cavity of the etherized host. Generally the operations were carried out with larvæ twenty-four hours prior to puparium formation (so-called "standard" age). When the fly had hatched, it was dis-

[1] Grateful acknowledgment is made to the American Philosophical Society whose grant-in-aid made the work herein reported possible.

sected in a 20 per cent solution of glycerin in alcohol, this medium being used to keep the bristles flexible and so prevent their breaking. After the various regions of the implant had been identified, it was then unfolded and examined under a compound microscope. If protected by a coverslip, such temporary mounts could be kept for weeks with an occasional addition of small quantities of the glycerin-alcohol mixture.

Another series of experiments was concerned with the injection of body fluid from prepupæ and pupæ into mature larvæ, prepupæ and pupæ. The technique used was similar to that of Beadle, Clancy and Ephrussi (1937) but differed in one respect. Newly-formed prepupæ were caused to adhere to slides by the application of a small amount of culture medium which acted as a glue. In this way it was possible to carry out operations without holding the pupa case. All injections were made posteriorly in order not to injure the macrochætæ-bearing regions.

Description of Stocks Used

The stocks were available from those maintained at Washington Square College, with the exception of the scute-1 stock which was obtained from Dr. G. P. Child of Amherst College. The characters of the various stocks used are briefly summarized below:

(1) + —— wild type, Woodbury and Oregon R strains. Bristle characters in the Woodbury stock somewhat variable, duplications and absences more frequent than in Oregon R.

(2) f (B) —— forked. Macrochætæ forked, gnarled or bent, the posterior scutellars showing the character in most extreme form. Microchætæ unaffected.

(3) sn^3 —— singed. Macrochætæ gnarled, curved and twisted, especially the posterior scutellars. Microchætæ also affected.

(4) ss —— spineless. All macrochætæ reduced in size, especially the scutellar, ocellar and post-vertical bristles.

(5) H^2 ext —— Hairless extreme. An extreme form of Hairless, practically all bristles and hairs missing. Only 50 per cent of the offspring H^2 ext. Stock maintained by constant selection. Trichopores (circular pits bordered by raised annuli) present.

(6) sc —— scute-1. Scutellar bristles nearly always missing, orbitals and post-verticals often gone, presuturals frequently absent. No trichopores present.

(7) y sc —— yellow scute-1. More variable than the above scute-1. One or more scutellar bristles found in about 35 per cent of the flies. Bristle color as well as body color yellow.

Experimental Data

Growth and Differentiation of Implanted Dorsal Mesothoracic Disks

The dorsal mesothoracic disk gives rise to the wing, the dorsal half of the thorax (except the humerus), and half of the scutellum (Chen, 1929). If both host and donor larvæ used are mature, well-developed bristles are found on the implant when it is dissected out of the newly hatched fly. The thoracic and scutellar regions of an implant are a light tan while the wing is gray. If allowed to remain in the host fly for a few days, the implant becomes much darker in color. Younger disks transplanted into hosts of "standard" age produce bristles which are not sufficiently well developed for a critical

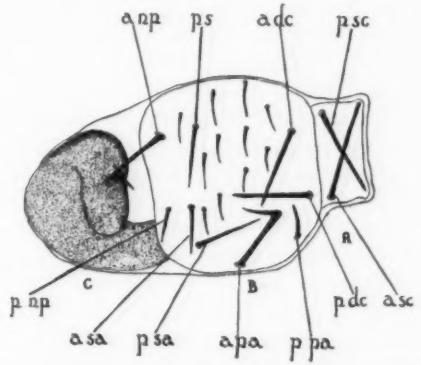

Fig. 1. Diagrammatic representation of a wild type implant showing bristle arrangement. Note the two bent bristles. The wing has been moved to one side and partially unfolded, but the thoracic region has not been touched. *A*, the scutellum; *B*, the thoracic region; *C*, the wing. The macrochætæ have been identified: *a sc*, anterior scutellar; *p dc*, posterior dorso-central; *p pa*, posterior post-alar; *a pa*, anterior post-alar; *p sa*, posterior supra-alar; *a sa*, anterior supra-alar; *p np*, posterior notopleural; *p sc*, posterior scutellar; *a dc*, anterior dorso-central; *ps*, pre-sutural; *a np*, anterior notopleural.

analysis to be made of the mutant character being studied. However, if such an implant be allowed to remain in the host fly several days after hatching, further development of the bristles will take place.

The implant on being dissected out of the host fly is found enclosed in a sac. Since the disk has not been everted during development, it should be carefully opened and spread out for critical observation of the bristles. The eleven macrochætæ developed by each dorsal mesothoracic disk include all the thoracic bristles except the humerals, which develop from the prothoracic disk (Chen, 1929). Size and arrangement of bristles with respect to each other and to the micro-

chætæ make it possible to identify each of them positively in a favorable implant (Fig. 1). If the action of bristle-removing genes, or of genes which cause the growth of extra bristles is being studied, a count of the macrochætæ is sufficient. The scutellar region is ordinarily easy to recognize, unless the scutellar bristles are absent as in scute-1 or Hairless extreme. In these mutants a study of the macrochætæ is essential in the identification of the phenotype of the implant.

Wild Type Disks in Mutant and Wild Type Hosts

Before the results obtained on implantation of bristle-bearing tissues into mutant hosts are reported, it is essential that certain factors which complicate the interpretation of the data be reviewed: It has been previously pointed out (Howland and Glancy, 1937) that wild type implants in wild type hosts exhibit a varying number of bristle types. One of these is simply bent, others strikingly resemble those of the mutants forked and singed, and still others are not identical with the bristle forms characteristic of any known mutant in *Drosophila*.

An analysis of the possible causes of this phenomenon indicated that these effects were the result of pressure exerted on the bristles during their development within the implant. It is evident that the effect of compression on the bristles must constantly be kept in mind, especially if bristle-bearing tissues are allowed to develop in mutant hosts such as forked and singed.

Wild type disks implanted into H^2 ext, ss, y sc, sc, f and sn^3 hosts are held to develop autonomously, since implants in f and sn^3 hosts produce no more forked and singed bristles than are found on the controls. Three or four of the larger bristles may show some distortion and one or two of the smaller ones may also be affected (Howland and Glancy, 1937).

The phenotype of all host flies was normal with the possible exception of a slight modification of the H^2 and y sc. In the former,[2] if the host on hatching proves to be H^2 ext, it is easy to say with certainty that the implant has had no effect, but in H^2 hosts the normal variability in bristle number leaves the matter open to question. Statistical treatment of large numbers would be required to determine beyond any doubt whether a greater number than the normal expectation had been induced to grow.

[2] It must be remembered that in this stock only 50 per cent of the offspring are H^2 ext, the other 50 per cent being H^2. (See description of stocks.) If used as hosts, the actual condition (H^2 or H^2 ext) can be checked on hatching. When choosing larvæ for donors from this stock there is no way to insure the selection of an H^2 ext mutant. On counting many hundreds of flies from the stock bottles it was found, however, that of the first flies hatching a far greater number were H^2. Larvæ used for hosts and donors were always chosen from the later "crop" in a bottle.

Spineless Disks in Mutant and Wild Type Hosts

The ss disks developed autonomously without modifying the phenotype of the host flies, H^2 ext, ss, y sc, sc, f and sn^3. No change in size of bristles was found on implants in wild type hosts, or any mutant hosts. Since the bristles in ss are smaller than in +, they are subjected to less compression in the implant. Therefore it may be stated with certainty that development is autonomous in every case. In all the ss implants examined but two abnormal bristles were found, and both of those were split from tip to base. Never were typical forked or singed bristles present.

Hairless Extreme Disks ^{2} in Mutant and Wild Type Hosts

Dorsal mesothoracic disks from H^2 ext stocks were implanted into ss, y sc, sc, f, sn^3 and + hosts without modifying the phenotype of the host. In every case trichopores were identifiable in the implant. In about 50 per cent of the implants some bristles were present, as would be expected.

Statistical treatment of further data would be required to determine whether the average bristle number is (*a*) less in H^2 implants in sc hosts and (*b*) greater in H^2 implants in + hosts, than in H^2 controls. In the first instance, genetic data would lead one to expect an intensification of bristle loss, for when H^2 is crossed to sc the total number of bristles missing is greater than the sum of those removed by the H^2 and sc genes (Sturtevant and Schultz, 1931).

Scute Disks in Mutant and Wild Type Hosts

This series of experiments was carried out with a thoroughly inbred sc stock in which a scutellar bristle is rarely found. Development of sc implants is autonomous in ss, sn^3, H^2 ext, f, y sc and + hosts. No scutellar bristles were found on any implants in this series. Host flies were normal with two exceptions which without further data cannot be considered significant. One wild type male host lacked an upper humeral and an ocellar bristle, while the left anterior scutellar was missing in one wild type female.

Yellow Scute Disks in Mutant and Wild Type Hosts

The development of y sc disks is autonomous in H^2 ext, ss, sn^3, f, sc and + hosts with respect to y character. This is also true of the sc character within the limits of variability which occur in the y sc stock. No modification of the host was observed. These results on yellow body color confirm those of Medvedev (1937) and show that yellow bristle color is also autonomous.

Forked Disks in Mutant and Wild Type Hosts

Development of f disks is autonomous in H^2 ext, ss, sc, y sc, sn^3 and + hosts without modification of the host phenotype. The f implants can readily be distinguished from sn^3 as the bristles are angular in f and curved in sn^3, although there is considerable overlapping of types (Howland and Glancy, 1937). In the particular f stock used, the microchætæ are straight, while in the sn^3 stock they are singed.

Singed Disks in Mutant and Wild Type Hosts

In every case development of sn^3 disks is autonomous, *i.e.*, in H^2 ext, ss, y sc, sc, f and + hosts, and the host flies are normal. The positive identification of sn^3 offers no difficulty since all the microchætæ as well as the macrochætæ show this character.

Injections of Mutant and Wild Type Body Fluid

Essentially the same series of experiments as those just reported were performed with the exception that injections of body fluid were made instead of imaginal disk transfers. In addition a wider range of ages of both host and donor was employed covering the late larval, prepupal and early (up to thirty-six hours) pupal periods. The injection combinations were made as follows: (1) prepupal fluid into mature larvæ and prepupæ, (2) pupal fluid into mature larvæ, prepupæ and pupæ. Larval fluid was not used.

In many instances the imagoes did not emerge, but had to be removed from the pupa cases. In some of these flies a few abnormal bristles, generally including one or more of the scutellars, were noticed. However, these results can be accounted for on the basis of compression of the developing bristles (Howland and Glancy, 1937). The genotype of the host from which the fluid was taken apparently did not influence the production of these abnormal bristles, for experimental control wild type animals exhibited similar anomalies.

Conclusions

Although there has been no indication of the presence of diffusible substances in ss, H^2 ext, sc, y sc, sn^3, f and + under the experimental conditions described, positive evidence might be forthcoming if (*a*) the entire range of time relationship between host and donor were tested, (*b*) if the amount of injected material were greatly increased or (*c*) if the implanted material were lodged in close proximity to the macrochætæ-bearing regions. The possibility that imaginal bristle-bearing tissues are impermeable to such substances or that they may

be unable to release them into the body fluid also deserves special consideration.

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THE EFFECT OF LIGHT ON THE VERTICAL MOVEMENTS OF ACARTIA CLAUSI (GIESBRECHT) [1]

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The changing vertical distribution of plankton Crustacea has long received a great deal of attention; copepods in particular have been the object of much study. Although many factors have been proposed to account for vertical migrations, it is now generally recognized that light is the chief instigator. The complexity of the affair, however, has been frequently discussed by such outstanding authors on the subject as F. S. Russell of Plymouth and G. L. Clarke of Woods Hole. The apparent lack of persistent responses makes it highly desirable to collect more facts dealing with the rôle played by light and it was with this end in view that the following studies were made on the marine copepod, *Acartia clausi*.

Field Studies

Field studies were carried out during the summers of 1935 and 1936, the collections of the first summer being confined to the head (Big Bay) while those of the second summer centered close to the mouth (off Bain's Island) of Inner Passamaquoddy Bay.

Methods

The two stations were established in regions devoid of any strong tidal currents which might complicate the results. The vertical distributions were determined under various conditions of light by collecting samples at four different depths for each condition of light.

The nets were made from No. 5 bolting cloth. By a special contrivance, it was possible to collect the organisms only at the specific depths; no such device was necessary for the surface tow. The arrangement for opening and closing the nets is shown in Fig. 1. The net is lowered to the specific depth by means of the line "a" which is kept taut, while line "b" runs out freely. In this way the net descends vertically due to the weight "w." On reaching the desired depth, a messenger is let down line "b" thereby releasing the attached mechanism. The net thus takes up the second position and line

[1] Contribution No. 183 from the Woods Hole Oceanographic Institution.

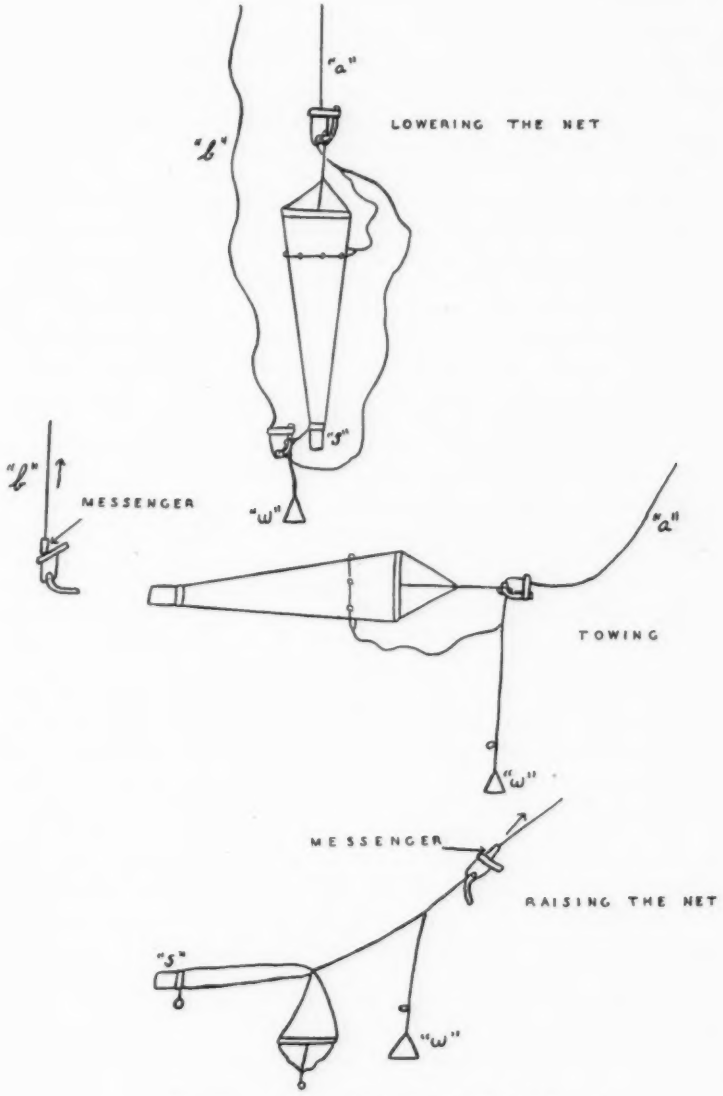

FIG. 1. *Closing mechanism used with the nets.*

"b" is withdrawn into the boat. The boat now slowly moves forward causing the net to strain the water. As nearly as can be judged, a definite and constant velocity of the boat is maintained. At the end of ten minutes a messenger is let down line "a" causing the net to be closed and the whole arrangement is withdrawn into the boat. The contents of the net are then carefully washed into the pint sealer "s," preserved in 4 per cent formaldehyde, and labelled.

Just before the nets were towed, the light intensity incident at the surface was measured by means of a solarimeter. This instrument is made by Kipp and Zonen, Delft, Holland. The light-receiving unit is a Moll surface thermopile covered with a hemispherical quartz window. The current produced is measured by means of a micro-ammeter. On a clear day this instrument will measure the intensity of sunlight from sunrise to sunset. The units of light intensity recorded are percentages of full noon June sunlight (F.N.J.S.), i.e. in percentage of the highest reading of noon June sunlight.

The transparency of the water was measured by means of a Secchi disk. It always disappeared from view at 16 feet.

The contents of the pint sealers were treated in the laboratory as follows: permitted to settle in a graduate to determine total volume; thoroughly shaken so as to produce a homogeneous mixture, and exactly 5 cc. removed by means of a Stempel pipette; the 5 cc. sample placed on a specially constructed counting cell, and the copepods identified and counted. At least two counts were made, except when there was so little plankton present that the whole tow was counted without making use of the Stempel pipette. The results of the counts were averaged. If the counts were within 10 per cent of their average, no further counts were made. The counts, when specimens were numerous, always showed close agreement. It was, however, often found necessary to make as many as six counts of the scarcer forms.

Each copepod was determined as being male, female, or young. For the purpose of identification, the following authors were consulted: (*a*) Sars—"Crustacea of Norway," and (*b*) Wilson—"Copepods of the Woods Hole Region." Such identification was not difficult as no very young forms were present in the tows, and the young found were sufficiently like the adults not to be easily confused as to species. The size, shape of fifth leg, antennule, and number of segments of the urosome were the characters mostly used for identification. The counting cell was so shallow that the copepods usually lay on their sides, thus exposing the fifth leg. A drop of dilute aqueous solution of methylene blue, added to the contents of the counting cell, greatly facilitated counting of the segments and recognition of the shape of the fifth legs.

Results

Consecutive Samples:—The results of a 24-hour series taken off Bain's Island on September 2 and 3, 1936, are shown in Fig. 2.

During the morning of September 2, the sunlight intensity was increasing rapidly so as to reach 60 per cent F.N.J.S. by 10 A.M. Males, females, and young were all absent from the surface with the young, on the whole, not as far down as the adults.

By 2 P.M. the intensity had increased to a maximum of 78 per cent F.N.J.S. Occasional clouds in the sky, however, cut the intensity down to about 28 per cent F.N.J.S. when crossing before the sun.

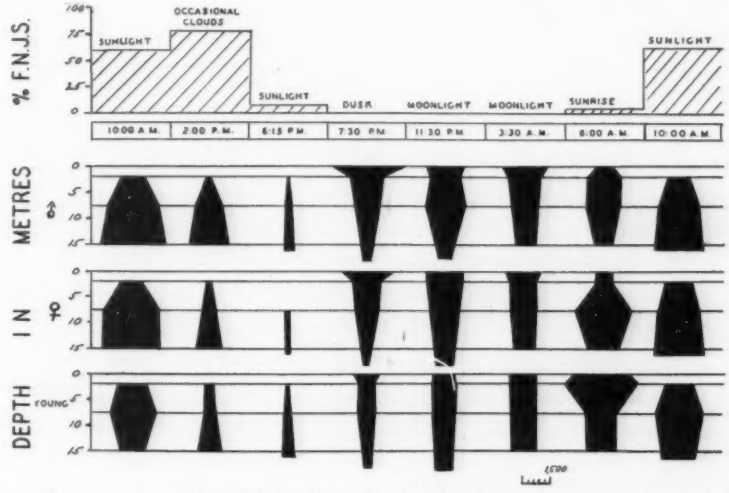

FIG. 2. The vertical distributions of males, females, and young over a period of twenty-four consecutive hours in September of 1936.

However, the prodominant light intensity was the maximum value recorded, and it is seen that males, females, and young are now in proportionately greater numbers at the bottom than before.

During the late afternoon, the sunlight was decreasing rapidly so that at 6:15 P.M. the light was 7 per cent F.N.J.S. Unfortunately, low numbers were captured by the nets at the time and some justification is felt for believing that the true picture of vertical distribution is not presented here.

A considerable drop in intensity occurred in the late evening so that by 7:30 P.M. a condition considered as dusk prevailed. The photometer would not register at this low intensity; the visibility was

very poor. During these conditions it is quite evident that a decided upward migration for males, females, and young had taken place so that decided maxima now occurred directly at the surface.

Shortly after 7:30 P.M. the full moon had appeared over the hills and by 11:30 P.M. visibility was good. Thus there was an increase in light intensity from 7:30 to 11:30 P.M. and on comparing the distributions at these two times males, females, and young alike appear to show a downward migration from the surface. It may be, however, that the moonlight had no effect and the animals were becoming uniformly distributed.

Moonlight prevailed throughout the rest of the night till 5:00 A.M. and tows taken at 3:30 A.M. indicate a decided tendency for uniform distribution of males, females and young. Thus continued moonlight does not result in their concentrating at any one particular depth.

At 6 A.M. the sun had risen over the hills causing an increase in light intensity as compared to the condition during the previous night. Males, females, and young all showed a downward migration from the surface with the young least affected, then the males, and finally the females. That is, the young showed a maximum at 2 meters, the males from 2 to 7½ meters, and the females at 7½ meters.

The sunlight continued to increase in intensity until by 10 A.M. it had reached 60 per cent F.N.J.S. Males, females, and young had descended still deeper into the water as compared with the condition at 6 A.M., with the males and females now showing maxima at the bottom and the young at 7½ meters. The young were still less affected by this condition than the adults. The vertical distributions are similar to those which obtained the previous day at 10 A.M. when the same light conditions prevailed.

Another series of consecutive tows was taken during the summer of 1935, the results of which are given in Fig. 3. During the late afternoon, the sunlight intensity was decreasing from 75 per cent at 2:40 P.M. to 27 per cent F.N.J.S. at 5:40 P.M. The two-meter tow taken at 2:40 P.M. was unfortunately destroyed. Nevertheless, it is evident that a decided upward migration had taken place for males, females, and young directly to the surface at 5:40 P.M.

The moon, which was two-thirds full, had been up all evening and so the light intensity was decreasing more slowly than had there been no moon. On comparing the vertical distributions of 8:55 P.M. with those which obtained at 5:40 P.M., it seems that a downward migration was taking place from the surface—possibly the result of the rate of decrease being so low as to no longer attract the animals

up to the surface such as occurred somewhat earlier in the evening, i.e. the rate of decrease must have been below their threshold (Clarke, 1930). It might have been, had the light continued as it was at 8:55 P.M., that the animals would later have become uniformly distributed. However, the moon set shortly before midnight so that the condition at 12:10 A.M. represents a decrease in light intensity. Here we have evidence of an upward migration in the upper layers to the surface—a migration particularly evident for the males and young.

During continued starlight conditions, males, females, and young tended to sink slowly in the water as seen by the condition at 3:20 A.M.

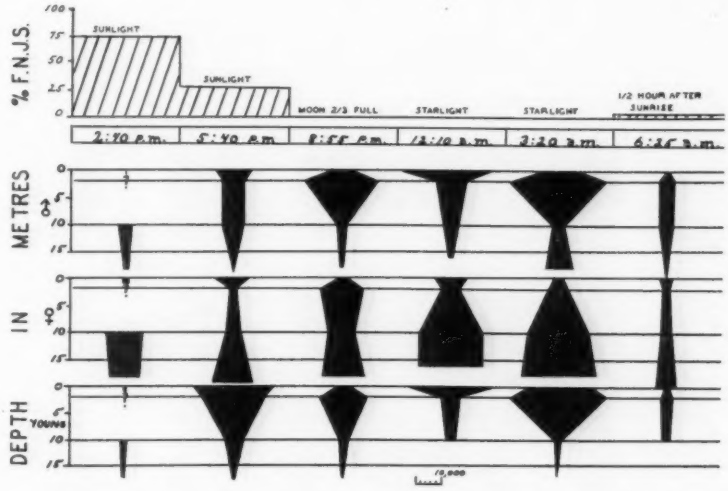

FIG. 3. The vertical distributions of males, females, and young during consecutive hours in August of 1935.

With the sunlight increasing in intensity in the early morning, reaching 3 per cent F.N.J.S. at 6:25 A.M., and comparing the vertical distributions with those at 3:20 A.M., it is quite evident that a great decrease in numbers had taken place in the upper levels. Although the animals appear to be more or less uniformly distributed, it is believed that a downward migration is in the process of being carried out, a fact which would have been made more evident had the tows been taken somewhat later (cf. Fig. 2 at 10 A.M.).

Occasional Samples:—From the above results it is seen that upward migration occurs under decreasing light, while downward migration

occurs under increasing light. Although similar vertical distributions were found to occur on different days when the light intensities were the same, it soon became evident that this is not always the case. Thus at sunrise on August 1 (Fig. 4), the animals were all farther down than at sunrise on September 3 (Fig. 2) even though the light intensity was the same on each occasion. However, the light intensities preceding sunrise were different on the two occasions; in the former case there had been no moonlight from 2:30 A.M. on, while in the latter

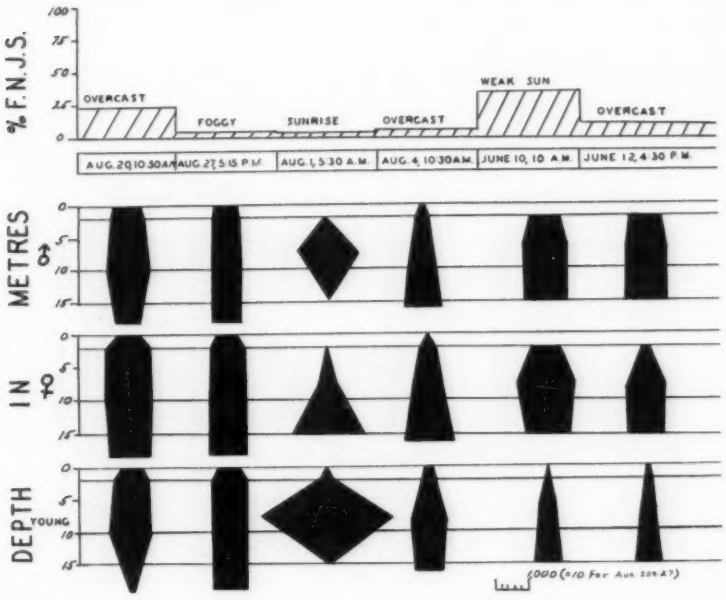

FIG. 4. The vertical distributions of males, females, and young as determined by occasional tows.

case there had been full moonlight all night until one-half hour before sunrise. In other words, following the greater increase in light intensity the animals descended farther down.

More examples indicating the difficulty of explaining vertical distributions on a basis of optimum intensity were found. For example, on June 12 at 4:30 P.M. males, females, and young showed practically the same vertical distributions as on June 10 at 10 A.M. (Fig. 4) even though the light intensities were decidedly different on the two occasions (10 per cent and 35 per cent F.N.J.S. respectively).

Increasing light apparently drives the animals down from the surface. When the light is steadily increasing in the morning, the higher the light intensity the farther down the animals are found (as has already been shown). However, this relationship between light intensity and depth does not necessarily hold when we compare the vertical distributions at the same time of the morning on two separate days having different intensities. This is well shown in Fig. 4. On August 20 at 10:50 A.M. males, females, and young were all, on the whole, nearer to the surface than at 10:30 A.M. on August 4, even though the light intensity was much higher on the former occasion (25 per cent F.N.J.S. as contrasted with 5 per cent F.N.J.S.).

Laboratory Experiments

Laboratory experiments, the results of which might prove to be of value in attempting to interpret the behavior of *Acartia clausi* in nature, were carried out in the basement of the Atlantic Biological Station during the latter part of September, 1936.

We have seen cases where reliable results showed that similar vertical distributions can take place under different light intensities. This fact was noted for males, females, and young alike of the same species and in the same locality. If the animals change their vertical distributions with changes of light intensity, is the direction of migration such as to bring them into a region of the same light intensity as that in which they existed formerly? Or, in other words, is there an optimum intensity for which the species is continually seeking? The very fact that similar vertical distributions can be found under different light intensities may answer the question in the negative in certain cases. However, to test the question somewhat more extensively, it was decided to study their vertical distributions under controlled conditions of light in the laboratory.

Methods and Results

Two tows were made off the Atlantic Biological Station wharf at St. Andrews at 4 P.M. during bright sunlight of intensity 50 per cent F.N.J.S. at the surface. The tows were made at the surface catching a few hundred *Acartia clausi*. Very few of any other species were present, *Acartia* being dominant in this region. In a quiet region it would be difficult to obtain *Acartia* at the surface under such conditions, but due to swift tidal currents they are forced up to the surface here.

Each tow was placed in a separate glass cylindrical glass jar of about 20 inches in length and 5 inches in internal diameter. Tar

paper was placed around and below each jar so that light could only come in from the top. A piece of thin glass was placed just above the open upper end of each jar so as to absorb heat from the electric light bulb when the latter was close to the jar. Over each jar was placed an electric light bulb of 50 watts, capable of being raised or lowered through a distance of 5 feet above the surface of the water in each jar. The arrangement was set up in a constant temperature room. The temperature of the water in the jars was thus kept the same as the water in nature from which the copepods had been removed (12° C.).

Effects of Single Changes in Intensity

Over one jar the electric light bulb was placed at a distance of 5 feet from the surface, while over the other a bulb was fixed a foot from the surface. The animals which had been obtained from the surface under bright sunlight were allowed ten minutes to distribute themselves and then the vertical distributions were determined by counting the numbers seen during a period of one minute through a square hole (1/4 inch square) in the tar paper. Such a count was made for each quarter-length of the jar. The resulting vertical distributions are shown in the following table:

	Bulb at 1 foot	Bulb at 5 feet
Section I (upper)	22	60
Section II	16	2
Section III	16	2
Section IV (lower)	1	2

The animals were subjected, as a result of the above procedure, to a decrease in light intensity in each case (sunlight intensity at the surface in nature was 50 per cent F.N.J.S., while a 50-watt bulb, when placed at a distance of one foot from the photometer, produced a reading of 4 per cent F.N.J.S., the intensity at a distance of 5 feet being 0.18 per cent F.N.J.S.). It is seen from the above that both changes in light intensity resulted in upward movement the extent of which was greater following the larger decrease in intensity. The individuals were uniformly distributed in the jars before being taken into the laboratory.

The electric bulbs were then left in the above positions until 2:30 P.M. the next day, and on examining the vertical distributions it was found that the individuals were uniformly distributed in each jar. The results point to a decrease in light intensity as the stimulus for upward migration and the greater the change in light the greater the upward movement. Furthermore, they indicate that the animals were not seeking an optimum intensity since they became

uniformly distributed when left for some time under the intensity in which they first moved upward.

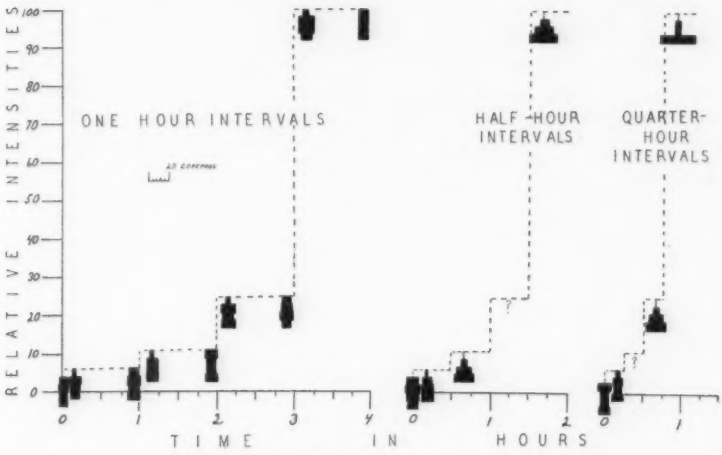

FIG. 5. The vertical distributions in glass jars resulting from increasing the light intensities at different frequencies.

The positions of the bulbs were then reversed so that the bulb previously at the surface was raised to 5 feet causing a decided decrease in light intensity, while the bulb previously at 5 feet was lowered to 1 foot from the surface causing an increase in light intensity. The vertical distributions ten minutes after the changes were made are shown in the following table:

	Bulb at 5 feet	Bulb at 1 foot
Section I (upper)	28	1
Section II	14	4
Section III	5	14
Section IV (lower)	1	30

The results show that a decrease in light intensity resulted in upward movement while an increase in light induced downward movement. The magnitudes of increase and decrease in both cases were the same and it is interesting to note that the resulting vertical distribution of the one is practically the inverse of the other. The bulbs were left this way until 9 A.M. the following morning and on examination a uniform vertical distribution was found in each jar. Thus it again appears that a change in light intensity is necessary to evoke migration of *Acartia*, while under static conditions no movement occurs.

Effects of Repeated Changes in Intensity

Increasing Light:—With the animals uniformly distributed and the bulb at 5 feet, the light intensity was increased by lowering the bulb 1 foot every hour, 1 foot every half-hour, and one foot every 15 minutes. The vertical distributions were determined shortly after each change was made, and in the case of one-hour intervals, readings were also made just before the next change. The results are shown in Fig. 5.

Decreasing Light:—With the animals uniformly distributed and the bulb at 1 foot, the light intensity was decreased by raising the bulb one foot every hour, one foot every half-hour, and one foot every 15

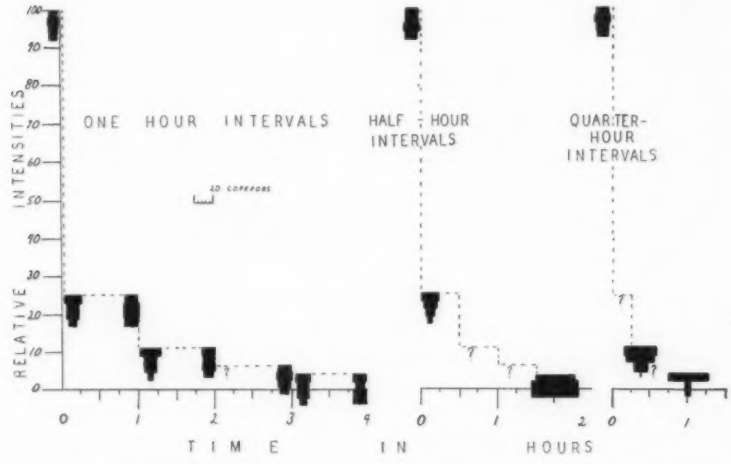

FIG. 6. The vertical distributions in glass jars resulting from decreasing the light intensities at different frequencies.

minutes. Readings were taken of the vertical distributions 10 minutes after each change was made, and in the case of one-hour intervals, readings were also made just before the next change. The results are shown in Fig. 6.

It is seen from the above two experiments, that on increasing or decreasing the light intensity by lowering or raising the bulb one foot every hour, slight but noticeable changes were produced in the vertical distributions of the copepods soon after each change was made, but that the animals returned to uniform vertical distribution before the next change was made. In the case of the half-hour intervals, the animals did not return to uniform vertical distribution but gradually

increased or decreased the distance at which the majority were found from the surface; the same applies to the 15-minute intervals except that the movements were more pronounced.

It is thus seen that when the light intensity remained the same for some time before the next change was made, the animals returned to uniform vertical distribution; but if the next change followed soon enough the animals continued to move either up or down, as the case may be, and the more frequent the changes the more pronounced the effect.

Examples are also seen where similar vertical distributions occurred under different light intensities, and also examples of different vertical distributions under similar light intensities.

Discussion

While the vertical distributions of *Acartia clausi* in nature were found to present some puzzling problems, it appears that some importance might be placed upon the direction and rapidity of change of light intensity, i.e., downward migration may be brought about by increasing light intensity while upward migration may be brought about by decreasing light, and some instances were found which suggest that the more rapid the change of intensity, the greater the response induced.

It is realized that the changes of light intensity produced in the experiment do not represent different rates of change but were merely different frequencies of change, and each change in itself was more sudden than usually occurs in nature (except possibly the sudden increase and decrease of light as the result of a cloud passing across the sun). Furthermore, it is realized that during any one series of changes the different magnitudes of change were in no two cases equal; thus the experiment does not represent the effects of similar magnitudes of change merely being applied at different frequencies. Taking everything into consideration, the results of the experiment are not strictly applicable to light changes as they usually occur in nature. Nevertheless, they do suggest that *Acartia clausi* requires *changes* in intensity to evoke vertical migration and the more frequently the changes occur the more the animals are stimulated to move either toward or away from the light as the case may be.

An interesting fact not already mentioned is that *Acartia* moved toward an electric light (or bright sunlight) after having been in darkness, while, as was shown above, they moved away from the bulb when the latter was brought closer to the animals after they had been previously in the light. Similar responses have been noted by L. B. Clark (1931) for the insect *Dineutes*.

Although it is felt that the facts presented in this paper by no means solve the manner in which light controls the vertical movements of copepods in the sea, it is believed that they are worth while considering before the very complicated problem of phototropism be solved.

ACKNOWLEDGMENTS

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ON THE EARLY DEVELOPMENT—BIPOLAR DIFFERENTIATION AND CLEAVAGE—OF THE ZEBRA FISH, BRACHYDANIO RERIO

EDWARD C. ROOSEN-RUNGE

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INTRODUCTION

Extensive studies of the early development of teleosts are very rare. The separation of the protoplasm from yolk and the first cleavages have been most thoroughly observed by Kowalewski (1886). Spek (1933) was the first to solve some of the problems connected with bipolar differentiation, especially from the point of view of colloid chemistry. The few morphological descriptions of the first events are for the most part simply qualitative. In only one paper, of His (1876), can exact measurements and a series of continuous quantitative observations be found. These, however, are little noticed in the literature, since they are reported as individual items without adequate statistical analysis.

The development of *Brachydanio rerio*, the zebra fish, a cyprinid from India, has never been described, and has been experimentally treated, as far as I know, only incidentally in a work of J. Oppenheimer (1936*d*), in which nothing is mentioned about the normal development of the egg. Of all the fishes whose development has been described *Cyprinus blicca* seems most like the zebra fish (v. Baer, 1835). From v. Baer's pictures it appears that the proportions of yolk and protoplasm and also the rate of development are almost the same. His discussion, however, begins with the blastula stage.

In some respects, *B. rerio* is an extraordinary fit laboratory object. Eggs in considerable number can be obtained every day of the year. The egg is transparent, has a small yolk and can be held in any desired position without trouble. Furthermore, the development is unusually rapid. The time from fertilization to hatching is about 60 hours at 27° C. Since the development presents several differences from the species already described and since so little is known about the earliest development of teleosts, it seems worthwhile to publish a simple description as a basis for further experimental study.

MATERIAL AND METHODS

The animals were kept in tanks of about 60 cm. length and 40 cm.

width with a water level of at the utmost 25 cm. In a corner, the bottom was covered with stones of 2–6 cm. in diameter, over which a dense clump of plants was placed. The most successful and probably most natural propagation will be obtained by keeping the fishes in schools of 12–15 specimens, of which 8–10 should be males to guarantee abundant fertilization. The most fertile age is from ¾ to 1½ years. The optimal temperature is 26–27° C., most conveniently held by a thermostat. If uniformly healthy eggs are desired, it is necessary to have an abundant artificial aeration and even circulation of the water. Under such conditions the zebra fishes spawn almost every day, but the same individuals do not always take part, particularly not the females. The spawning occurs in the early morning at about 8 o'clock in winter, in summer often as early as half past five. The fertilized eggs drop into the plants and among the stones. The parents are not able to get the eggs to eat them, though they try continuously to find them.

The eggs were caught with a dip-tube. Occasionally I fished them up while they were sinking and was thus able to observe the very early stages. Usually they were pipetted into small dishes, which were wrapped in gauze and placed in the aquarium near the aerating equipment. It is easy to obtain 10–50 eggs daily during the first 3–4 weeks of spawning. Later the number decreases and there occur intervals of 1–4 days. With copious and varied food, research material is available almost continuously, as the young ones spawn at 5–7 months of age and the seasons have practically no influence on the fertility in the aquarium.

Observations

Most observations were made on the living egg. Unless especially designated as averages, the measurements in this paper are all taken from two single eggs, the development of which has been recorded in a film. This film was taken with time accelerator under the microscope, and a partial description of it has already been published (Roosen-Runge, 1936). It is necessary to emphasize that the greatest advantage of such a film for science is not in looking at it, projected on the screen, though that may show an interesting survey. Careful measurements combined with comparison of the running film taught me to appreciate the film as a research method. Especially for the rapid events before the first cleavage measurements gave much better information than the observation of the running pictures. I used a copy of small size (16 mm.) from the standard sized negative and measured picture by picture with an eye-piece micrometer under the

low power of the microscope. For the most important parts I had pictures of two different eggs to work with. While taking the film the eggs developed under a constant temperature of 27° C. The pictures were done every 1½ second corresponding to a 24 times acceleration. The film was financed and assisted by the Deutsche Gesellschaft für wissenschaftliche Filme m.b.H., Berlin.

The Development

The following description will deal only with the development of the egg after fertilization. The mature egg of *B. rerio* is white and nearly opaque, homogeneously filled with granules. The average diameter is about 625 μ. When the egg leaves the oviduct it is apparently not so completely differentiated as the eggs of most teleosts. For example, in *Corregonus macrophtalmus*, *Salmo irideus*, *Squalius cephalus*, *Leuciscus rutilus*, *Barbus fluviatalis*, *Tinca fluviatalis* and *Gasterosteus aculeatus*, it was observed by Spek (1933), that a hydration of the cell substances has already taken place in the ovary, producing a much more transparent appearance in the eggs, and that in all these species bipolar differentiation is obvious before fertilization occurs. In the ripe egg of *B. rerio* there is no visible separation of the blastodisc. This does not mean that no differentiation exists. The polarity and the later bipolar differentiation is foreshadowed by the location of the nucleus and the very small amount of protoplasm around it. When the egg is laid, the water causes only a slight concentration of the protoplasm. This had been previously observed in the herring (Kupffer) and in the perch and the pike (Lereboullet), but most teleost eggs show in the ovary a distinct blastodisc, the increase in size of which is strongly accelerated by the contact with water.

As I was never successful with artificial fertilization, I cannot report anything about the first minute of development from direct observation. My earliest observation begins about a minute after fertilization. The film, for which the egg had to be brought into position, does not start before 1½ minutes, and so the exact measurement does not begin earlier. During the first minute the egg is already changing rapidly. Due to the fact that it is not completely differentiated in the ovary, all the first events are condensed into a very short time, so that during the first 15 minutes many more things happen than during the first 15 hours in the development of the trout (this is in both cases until the time of the first cleavage).

Before entering into a detailed description of these early events it is necessary to say something about the method of orienting the egg. There is a polar axis in the mature egg of the zebra fish just as in

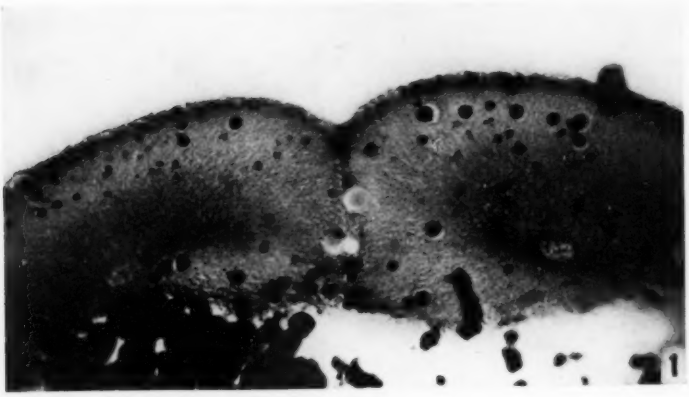

FIG. 1. Section through two cells of a 4-cell-stage. Nuclei in early prophase. Stained with Heidenhain's hematoxylin. 8 μ. × 810.

FIG. 2. Section through an egg during early separation process. Yolk broken. The protoplasmic coat is visible around the cell, with a slight concentration at the vegetal pole. Stained with Heidenhain's hematoxylin. 8 μ. × 115.

other teleosts, though it is less conspicuous. Immediately after the membrane is lifted off (Fig. 1), the egg appears somewhat oval in shape with the long axis as the polar axis and the animal pole marked by a little depression, where the sperm entered. But it is far more important for the orientation of the egg that, in addition to this, the direction of the first cleavage plane seems also to be established very early. The cavity on the animal pole is from the first moment of observation slightly elongated, so that it appears deepest when seen along the long axis. It is therefore possible to orient the egg during the earliest stages by turning it around the polar axis, until the cavity appears most excavated. If this is done (Fig. 1 in this paper, Fig. 1*a* in Roosen-Runge, 1936) it is almost certain that the first cleavage plane will appear perpendicular to the plane of observation. Figure 7 shows an egg in the fifteenth minute that had been exactly oriented in the described way. In Fig. 11 the first cleavage of the same egg can be seen. The direction of the first cleavage plane can be foretold at least shortly after fertilization. It remains a question whether or not it is established by the direction of the sperm entrance. If so, the determination must take place in the very short time of about one minute.

About one minute after fertilization the egg has a diameter of about 750 μ from membrane to membrane. It is not quite round but the deviations are usually small. The membrane is soft and very extensible. Spek discovered that in teleosts it consists of the primary membrane of the oöcyte and the radially striped cortical layer clinging to it. The external protoplasmic layer of the oöcyte takes up water and lacunæ appear, which later flow together and establish the perivitelline space. Still later there begins an enormous secondary enlargement of the space, because water has been attracted into it. But in *B. rerio* some material for this enlargement evidently comes from the egg itself during the first few minutes, since the cell shrinks at first, though the egg as a whole is swelling. After the second minute the cell is about 580 μ in the equatorial diameter and 605 μ in the polar diameter. One and a half minutes later (Fig. 2) the equatorial diameter is still the same, 580 μ, but the polar diameter has decreased to 565 μ, which means that together with a pronounced change in shape, the egg shrank and lost material into the space. The shrinking of the cell takes only a very short time of about 3½ minutes. At the end the cell appears more round (Fig. 2) but the pole of the faintly visible blastodisc is somewhat more flattened. The diameter from membrane to membrane has increased from 750 μ to 830 μ. During the following 2 to 3 minutes the cell itself increases greatly in volume, probably due to an increased hydration of its substances.

PLATES I AND II

Explanation of Figures

Photomicrographs of living zebra fish eggs, taken from a film. Development at 27° C. × 19.

FIGS. 1–6 present one egg in the different stages. The egg is oriented so that the first cleavage plane will occur perpendicular to the picture plane. The egg turned between Figs. 5 and 6, so that only a small part of the blastodisc is visible in Fig. 6.

FIGS. 7–23 present another egg. The orientation is exactly the same as in Figs. 1–5.

PLATE I

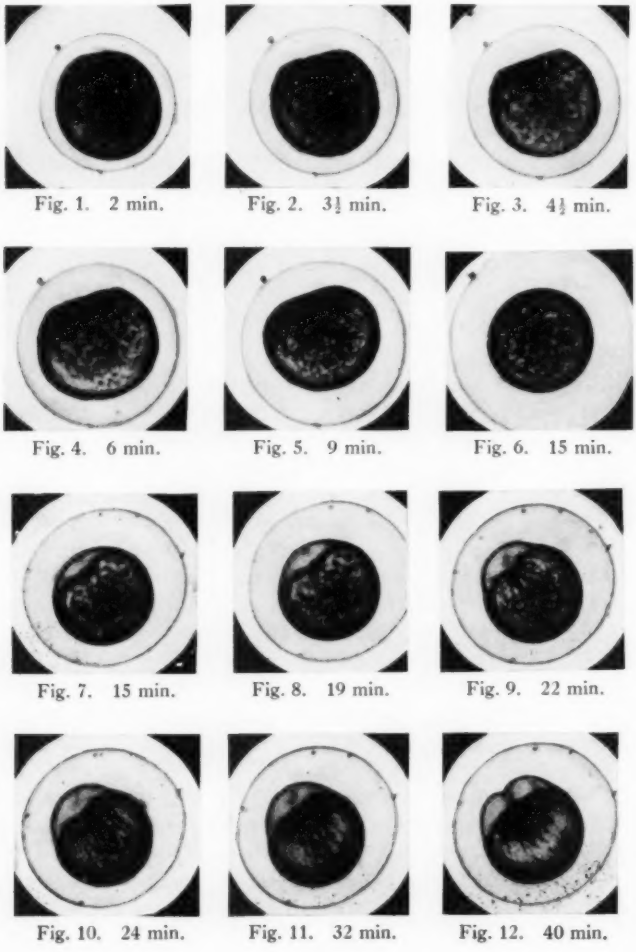

Fig. 1. 2 min. Fig. 2. 3½ min. Fig. 3. 4½ min.

Fig. 4. 6 min. Fig. 5. 9 min. Fig. 6. 15 min.

Fig. 7. 15 min. Fig. 8. 19 min. Fig. 9. 22 min.

Fig. 10. 24 min. Fig. 11. 32 min. Fig. 12. 40 min.

In describing the following change in shape the diameter of the first cleavage plane is referred to as the frontal diameter; the sagittal diameter is that of the second cleavage plane. These terms have no reference to the future planes of symmetry of the embryo. The longest diameter is always used. The stage in Fig. 4 has a polar diameter of 575 μ but a sagittal diameter of 665 μ. At this time the sagittal diameter is 15–25 μ longer than the frontal diameter. The egg is somewhat elongated, as though it were foreshadowing the direction of the spindle axis of the first cleavage which is to follow. The elongated shape disappears entirely from the fifteenth minute until it reappears about the thirtieth minute at the time of the first cleavage. An elongation is observed as the first cleavage spindle develops in most eggs, but the previous lengthening is unusual and the relation of the two is not clear at present.

At about the fifth minute the change of the cell substances is very conspicuous. The cell becomes more transparent in a short time beginning at the vegetal pole. If mixed with water the substances coagulate in a much finer and less granular form than if such a mixture is made during the first minutes. They are probably changed in their colloidal condition by a strong hydration. The enlargement of the cell is greatest at exactly the time when the animal pole is most flattened, and from exact measurement it can be seen that the increase in volume of the whole egg, that is the taking up of water through the membrane, begins to slow down at the same moment. It can be seen in Fig. 4 that there is a very thin, clear protoplasmic coat around the cell in direct connection with the blastodisc. This coat has gradually appeared during the preceding minutes. It shows an uneven outer surface which changes its shape continuously. The movements of the protoplasm are comparable to the so-called amœboid movements, observed in the trout shortly after fertilization (Oellacher, 1872). The protoplasmic layer is very distinct for 2 to 3 minutes and then begins to disappear with the next changes of the egg until it is entirely invisible in the ninth minute (Fig. 5). The cell rounds more and more and the blastodisc, flattened to a thin and even layer, becomes also round and higher with a smaller base. There is a conspicuous enlargement of the blastodisc now for the first time. Not the slightest streaming which would indicate a separation of protoplasm from yolk is visible, but the protoplasmic cover has become so fine that it is traceable only in the sections. The concentration of protoplasm into the blastodisc, at this time, seems to be an entirely different process, from what will be presented later as the separation of protoplasm from yolk. An actual separation must have happened earlier when

the clear protoplasmic layer appeared around the cell. At the time of the rounding of the egg the protoplasm concentrates from this layer into the blastodisc and thins out consequently. This phenomenon is unusual and seems never to have been described.

After the stage in Figs. 6 and 7 is reached, there is an obvious break in the development, which is marked particularly by the fact that no visible changes happen during the next 5 minutes. No movements of yolk substance, nor of particles inside the protoplasm are visible. The cell is exactly round; all diameters are about 605. Shortly before the spherical shape was attained, the swelling of the egg as a whole had ceased, and the diameter of the egg remains constant from now on till the embryo hatches. That means that the volume of the egg does not change any more and that the membrane no longer expands. The egg finally has a diameter of 1 mm.

The first movement inside the yolk is to be seen at about the fifteenth minute after fertilization. At first it appears as a fluctuation in the granular mass without a prevalent direction, but there must be a directed migration of material, since the blastodisc is already growing. The area between yolk and blastodisc, the "Mischzone," which appears as a dark refracting zone in Fig. 7, becomes smaller until after 7 minutes it has almost disappeared. At that time (Fig. 9) conspicuous lines become visible in the yolk, caused by a directed streaming of protoplasm into the blastodisc. The material comes, at first, not so much from the center of the yolk, but flows more vigorously from the superficial parts, particularly from those near the blastodisc. The lines converge towards the center of the blastodisc, where they form acute angles. Two dark areas which appear symmetrically in the blastodisc at the base mark the location of the nuclei. The higher density of the protoplasm around the nuclei can also be observed in the stained sections (Fig. 1 in the text). When the position of the nuclei first becomes evident in the living egg, the darker areas around them are apparently distorted by the streaming of the protoplasm. The view from the animal pole confirms this, showing that the stream comes onto the blastodisc through the central area of the base, then spreads towards the upper part of the cell surface like a fountain. The most superficial layer is not involved in the movement. Granules appear anywhere throughout the cell, except in the immediate vicinity of the nucleus. Some of them lie in the surface layer; they do not take hematoxylin and look faintly yellowish in the sections. These cannot be distinguished from the stainable granules in the living egg.

At about the twenty-second minute the direction of the stream begins to change. The lines in the yolk appear more nearly vertical

and very soon almost perpendicular to the base of the blastodisc. At this time the "Mischzone" begins to grow rapidly, reaching its climax when the cell is cleaving. It represents a very dark and wide zone, which is situated under the central region of the blastodisc (Fig. 11). It apparently consists of fine granules mixed with larger yolk drops; between the two substances exists a great difference of refraction (Spek, 1933, p. 522). I was able to observe in the running pictures at this time a distinct stream of numerous fine granules in the protoplasm, apparently in the layers immediately underneath the surface. The direction of this stream is opposite to that of the axial stream in the yolk, but the velocity is about the same. It is visible only against the background of the contrasting yolk drops and not in the blastodisc itself. It seems probable that the concentration of protoplasm at the vegetal pole, which has been observed by Kowalewski (1886) and can be easily seen in the sections of *Brachydanio* eggs at this stage (Fig. 2 in the text), is only a consequence of the counterstream in the protoplasmic coat. The fact of this counterstream is of great importance, particularly with respect to the observations of Spek (1933, p. 524). He never saw any counterstream in teleost eggs and therefore denies that a lowering of the surface tension at the animal pole could account for the streaming of the protoplasm in that direction. I myself, though I knew the fact from the film, was never actually able to see it in the living cell. Only by means of speeding up the movement was it possible to overcome the great difficulty of distinguishing the fine transparent protoplasmic stream from the coarse movement in the yolk.

The first cleavage is indicated by a slight depression at the animal pole at about the thirty-second minute (Fig. 11). A few minutes later the cells are divided by a line but not separated from each other (Fig. 12). The division is almost complete and no protoplasmic connection can be observed at the base of the cells as in most other teleosts, for instance, in the trout. The border can be stained with Heidenhain's hæmatoxylin as a very fine but distinct line (Fig. 1 in the text). As the blastomeres separate, the "Mischzone" between yolk and blastodisc decreases, passing a stage where it seems to consist mostly of large distinct granules (Fig. 13, Plate II). In as much as the nuclei are usually divided at the twenty-second minute, the time which it takes to complete the cleavage seems relatively long. After the cleavage is completed (Fig. 14) the cells lose their spherical shape again and show a wide plane of contact. The streaming of the protoplasm, which ceased after the cells were completely rounded, begins in the parts nearer to the blastodisc as it did the first time and

PLATE II

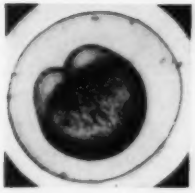

Fig. 13. 47 min.

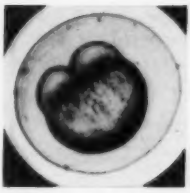

Fig. 14. 51 min.

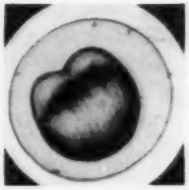

Fig. 15. 65 min.

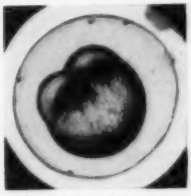

Fig. 16. 67 min.

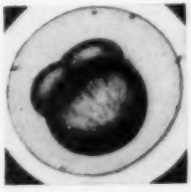

Fig. 17. 80 min.

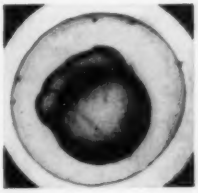

Fig. 18. 92 min.

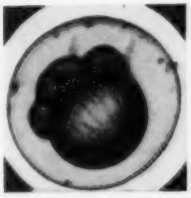

Fig. 19. 104 min.

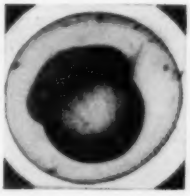

Fig. 20. 117 min.

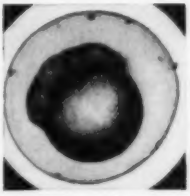

Fig. 21. 128 min.

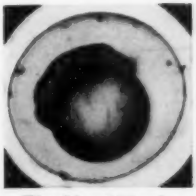

Fig. 22. 136 min.

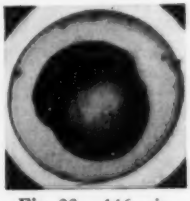

Fig. 23. 146 min.

gradually increases till the stream comes from all parts of the yolk (Figs. 15 and 16), but the stream is not as vigorous, of course, because the amount of protoplasm left in the yolk is very much smaller.

The "Mischzone," which appeared not very voluminous when the first cleavage was completed, becomes more conspicuous again. The climax of the separation process and the formation of the "Mischzone" is reached when the second cleavage plane becomes visible, perpendicular to the first one (Fig. 16). During the further cleavages this cycle can always be seen to a gradually decreasing extent: the cells lose their round shape when the "Mischzone" and the stream into the blastodisc is minimal; they appear adherent to each other with wide contact areas, when the volume of the "Mischzone" and the stream are at their maxima, and at this moment the cleavage plane becomes first visible (Fig. 18). Then the cells round again, become almost separated and the streaming discontinues (Fig. 19). The stage of 16 cells is to be seen in Fig. 20. The cells are rounded, so that three of the four rows of cells, one behind the other, can be distinguished in the picture. The same cells appear flattened as they divide into 32 (Fig. 21). They have rounded again in Fig. 22, which shows the cells on the outside more spherical, because they are not as widely adherent to surrounding cells. At the time of the first tangential cleavage the cells are again more flattened and the outline of the blastodisc appears smooth (Fig. 23).

The first cleavages in *B. rerio* always appear in the same order and show in every respect a considerable constancy. The type of cleavage is the same as in *Fundulus heteroclitus*, recently described by J. Oppenheimer (1936*c*, p. 408). It may be noted that among the teleosts described in the literature there are some with a very variable cleavage plan, e.g., *Siphostoma Floriaæ*, *Serranus atrarius*, etc. Others have a very regular and invariable cleavage pattern, e.g. *Fundulus* and even more so *B. rerio*. The second cleavage plane appears perpendicular to the first one, the third parallel to the first, the fourth parallel to the second and the fifth parallel to the first. The sixth cleavage is the first horizontal one. In *Fundulus* the fifth cleavage is usually horizontal. The cells divide synchronously for a long time. In the film a rhythm of division can be seen still in the blastula stage. This phenomenon will be followed into details in later work.

Discussion

The most obvious point of view, from which the striking changes in the early development of the egg, in volume and shape, refraction and streaming rate may be surveyed, is that of the physical state of

the cell, but it is a question how far the physical state can be determined from simple observations without experiments. It is *a priori* certain that a quantitative analysis is quite impossible in this way and that many delicate changes which occur in viscosity and surface tension do not appear as changes in shape, or in refraction, or in the rate of movement. But if we confine ourselves to the most striking visible changes, it may be possible to conclude something indirectly. The results concerning physical changes in the living egg obtained by experimental methods, microdissecting and centrifuging, are not so abundant and unquestionable that an attempt to confirm one or the other by different means would be superfluous.

Fertilization initiates a complex of processes. At first, the membrane lifts off, and an intake of water occurs. Then the egg begins to shrink and the dark granular structure of the unfertilized egg becomes even more opaque. When fixed in this stage there appear clouds of fine granules just outside the surface of the cell in the perivitelline space, and under the influence of the fixing fluid, more of the same stuff will be pressed out of the cell, as it shrinks visibly. Though faint traces of substance appear in all stages in the perivitelline space after fixation, no material is observed to come out of the cell except in the very first time after fertilization. The intake of water is at first confined to the perivitelline space; the cell itself loses some kind of albuminous substance into the hypotonic fluid, which begins to surround it. Whether a change in the colloidal state of the egg cytoplasm is involved in this process cannot be told. Very soon, however, there seems to be more evidence for an actual change. The cell begins to swell, the surface shows amœboid movement, a clear protoplasmic coat becomes visible and the content of the cell as a whole becomes more transparent. The granular structure appears coarser now. The most striking change is the flattening of the animal pole. When this occurs, the blastodisc is no longer a faintly visible concentration of granular protoplasm, but consists in a distinct clear disc. The whole animal pole, including both the yolk part and the cytoplasmic blastodisc, is flat and the yolk keeps the same flattening at the base of the blastodisc throughout the separation process and the first two cleavages, though the flat surface of the blastodisc disappears quickly. Later the yolk becomes a spherical body until the blastula stage when it again flattens, at the base of the blastodisc.

The next change is due to the hydration of the cell itself, which swells and becomes more transparent. This hydration seems to be analogous to the hydration observed in most eggs after fertilization. At this time, the last polar body is extruded, and the fused pronuclei

probably come to a brief pause, as has been observed in other teleosts. The egg shows a complete resting stage before the first cleavage and the separation of protoplasm from yolk begins.

There are apparently three stages to the bipolar differentiation in the development of the zebra fish egg. At first the bipolar differentiation is indicated by the situation of the nucleus. The processes of fertilization and the following hydration bring about the concentration of a certain amount of protoplasm around the nucleus and so the blastodisc is established. A further concentration of protoplasm in the blastodisc occurs at the beginning of the first mitosis, and the process of separation comes to a rest, when the nuclei are completely divided and in a resting stage. It begins again with the prophase of the succeeding mitoses. This points to a connection between the two processes. The connection is to be sought in all probability in the physical state of the cell, which is altered during the cell division. Spek (1933) tried to determine the cause of bipolar differentiation in teleost eggs by means of his ingenious experiments using indicators on the living egg. He proved that gravitation is not a possible cause for the separation and considered surface tension likewise impossible, since the surface tension theory (Spek, 1918) requires a counterstream corresponding to the axial stream, and no such counterstream was observed. He tries to explain the bipolar differentiation chiefly by means of electrical forces, which are exerted between the different particles of the colloidal mixture. I do not have any reason to doubt this result, but in the *Brachydanio* egg it seems evident that the physical state of the cell, viscosity and surface tension, have also an important influence on the separation process. The main argument against this loses its value, because a counterstream, which has the opposite direction of the axial stream of protoplasm into the blastodisc, is actually present. In addition, there is certainly abundant indication that there is a change in viscosity and surface tension taking place at the time when the separation process begins. The initial change in the physical state starts with the hydration of the cell; the later ones begin with the cell divisions.

The changes which the cells undergo during mitosis are obvious in the living egg, though no nuclear structures are visible. The separation process is always at its climax when a cleavage plane first becomes visible in the late anaphase, it decreases rapidly while the cells round up, and the cleavage is completed, and it begins again very soon, probably with the prophase, when the surface tension of the cells seems to be lowered. The phenomenon of the "Mischzone" is not easy to explain. Whether the great differences in the volume

and density of the area underneath the blastodisc are due merely to an alteration of the size of the particles in the emulsion with the changing rate of movement in the substances, or whether there is an actual change in index of refraction of the protoplasm, remains a question. In some respects the last conception seems more probable, because sections show only insignificant differences in size of the particles in the "Mischzone." Flemming (1891) found that connective tissue cells are dark in the earlier stages of mitosis and became lighter in the late anaphase. The "Mischzone" of *B. rerio* eggs appears darkest just at the anaphase.

Chambers (1920), in the course of his experimental study of the consistency and viscosity of the sea urchin egg, found that the shape of the blastomeres, round in some stages, more flattened in others, fitted well his conclusions concerning the viscosity changes as obtained with the micromanipulator. The changes in the shape of the cells of the zebra fish egg are also very striking, as has been described, and they are also immediately connected with the general physical state of the cells. Definite conclusions, however, as to the viscosity and surface tension from the shape of the cells alone would be vague without a quantitative investigation.

Summary

A detailed description of the early development of the egg of *B. rerio* is presented. The observations are made chiefly on the living egg. The measurements are made on a moving picture, taken by time accelerator. This proved to be a valuable help in discovering facts which could not be detected by simple observations.

After fertilization the egg as a whole takes up water, but the cell itself shrinks. Later the cell itself begins to swell and to take up water.

The bipolar differentiation is first indicated by the position of the nucleus and a small amount of protoplasm around it. A conspicuous separation of protoplasm from yolk begins when the cell first takes up water and ceases during a complete resting phase before the first mitosis. With the beginning of the first mitosis a separation process of different nature takes place. This consists in a streaming of protoplasm through the yolk towards the blastodisc. In addition, a counterstream towards the vegetal pole is clearly visible in the film. The changes in the physical state of the cells connected with the mitoses are immediately correlated with the rhythmic separation process.

It is possible to orient the egg at the time of fertilization not only with respect to the polar axis but also with respect to the first cleavage plane.

I am deeply indebted to Professor J. W. Wilson, Brown University, for having frequently discussed the paper with me and given valuable advice regarding its form.

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THE EFFECT OF ULTRA-VIOLET LIGHT UPON CLEAVAGE IN CERTAIN MARINE EGGS

I. Some Studies on Cleavage Rate

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Introduction

Results of studies on the biological effect of radiation are reported extensively in the literature. The action of radiations of short wave length is reviewed by Packard (1931), and a splendid summary of radiation effect on living protoplasm is presented by Heilbrunn and Mazia (1936). A widespread therapeutic application of the biological action of radiation is discussed by Mayer (1925), Saidman (1925), Bach (1927), and Guillaume (1927). In the large body of experimentation upon the subject, cellular sensitivity has an important rôle in the analysis of radiation effect. Frequently this sensitivity has been regarded as susceptibility or resistance during cellular activity. It is indicated by the alteration of the reaction time of biological processes whose end-point is some well-defined morphological change which is characteristic of the normal activity of the cell. The first cell division in fertilized eggs of certain marine invertebrates is the process which has been selected as an experimental criterion by many investigators. Packard (1916) showed that cleavage rate in eggs of *Arbacia* was accelerated following exposure to radium emanations. The alteration of the rate of cell division (usually a delay in the time of cleavage) by X-rays was reported by Richards (1914) for eggs of *Planorbis;* Langendorff (1931) for eggs of *Psammechinus;* Henshaw (1932) for *Arbacia;* Henshaw, Henshaw, and Francis (1933) for eggs of *Chætopterus*, *Nereis*, and *Cumingia;* Heilbrunn and Young (1935) for *Arbacia;* and Henshaw and Francis (1936) for *Arbacia*. A similar effect by exposure of eggs to ultra-violet light is shown by Lillie and Baskervill (1922) for *Asterias* and Chase (1937) for *Urechis*.

The present work details the effect of ultra-violet radiation upon the rate of cleavage in eggs of the sand dollar, *Dendraster excentricus*, and the echiuroid worm, *Urechis caupo*. The experiments are for the

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purpose of determining the sensitivity of marine eggs to radiation effect when exposures are made at certain stages in development during the period from insemination to first cleavage.

Procedure and Results

The two forms, *Dendraster excentricus* and *Urechis caupo*, are abundant in the Monterey Bay region of the California Coast.

Table I

Stages during pre-cleavage development when eggs were irradiated

Period	Description of stages	
	D. excentricus	*U. caupo*
Before insemination	The process of maturation is completed.	The process of maturation has not begun.
15 minutes after insemination	The large sperm aster is seen and the pronuclei approach each other.	The germinal vesicle breaks down, indicating the beginning of the process of maturation.
30 minutes after insemination	Fusion of the pronuclei.	The first polar body has been extruded and the formation of the second polar body is taking place.
45 minutes after insemination	The resting nucleus stage which precedes the mitotic phases.	The second polar body has been extruded and the process of maturation is completed.
55 minutes after insemination	Phases of mitosis which lead to first cleavage.	
60 minutes after insemination		The resting nucleus stage which precedes the mitotic phases.
70 minutes after insemination		Phases of mitosis which lead to first cleavage.

Animals were collected and kept in aquaria supplied with well aerated, running sea water. Experiments were arranged so that eggs and sperm were obtained from freshly collected specimens. For an experiment, a quantity of gametes was used which showed high fertilization capacity (98 per cent to 100 per cent development of fertilization membranes). Eggs were placed in finger bowls containing 250 cc. of sea water and dry sperm were kept in covered Syracuse dishes. A sample from the supply of eggs was placed in sea water to a depth of

2.5 mm. in each of eight thin-walled, flat-bottomed glass dishes and exposed to ultra-violet light for durations of 15, 30, 45, 60, 75, 90, 105, or 120 seconds. The eggs in each dish then were inseminated with a standard sperm suspension (a drop of dry sperm from a micropipette added to 5 cc. of sea water) and allowed to develop in a greater volume of water. A larger sample was taken from the supply of eggs, inseminated, and allowed to develop. As the eggs reached a certain stage in development (see Table I), they were placed in each of eight dishes as in the previous case and given a duration of exposure. The irradiated eggs then were allowed to continue to develop in a larger volume of water. A batch of eggs which served as the control was taken from the general supply of eggs and inseminated simultaneously with each sample of eggs which was given the treatment with the ultra-violet light. The time of insemination in controls and rayed eggs was recorded.

Caution was observed in handling eggs and their contamination was guarded against so as to insure that premature development did not occur. Experimental procedure was standardized so as to minimize the possibility of aberrant results. The gametes, from the time of their removal from the animals to the end of an experiment, were kept at a regulated temperature of approximately 20° C. Before exposures the ultra-violet lamp was allowed to burn 10 minutes and traces of ozone were removed. During exposures dishes of eggs were placed at a distance of 30 cm. from the source of the light and the eggs were exposed to the full spectrum of the mercury arc. Radiations were made with an Analytic Model Quartz Lamp, Hanovia, which operated on a 110 volt circuit—alternating current—60 cycles—five amperes. Approximations of the intensity of the output of ultra-violet radiation at the distance were made frequently during the experiments and the variation detected was negligible.

The first three cleavages of controls and irradiated eggs were observed and the time of each was recorded at the moment when the reacting half of 10 eggs had begun to divide. Ten recordings were made for each sample of eggs, both rayed and controls, in order to obtain the time of the cleavage of the reacting half of 100 eggs for each. These observations were repeated nine times during the course of an experiment. Twelve experiments were run for each species so that the data are based upon the reacting half of 12,000 eggs at each exposure. Since the experimental criterion was the time of 50 per cent cleavage no data were included for eggs which showed a lower percentage. In all cases measurements on irradiated eggs were rejected when the controls failed to show 98 to 100 per cent first cleavage.

Measurements of the actual time from insemination to a particular cleavage are given for eggs of *Dendraster* and *Urechis* respectively, in Tables II and III. It is revealed clearly that the time-lapse from insemination to first cleavage in eggs of both species increases with increase in length of exposure, followed by a similar effect on the rate of the subsequent two-cell divisions. Delay in segmentation time occurs regardless of the stage in development when eggs were rayed, being greater at longer exposures for certain stages than for others. The exception is to be found when eggs of the sand dollar were rayed just before first cleavage.

TABLE II

Average time-lapse (minutes) from insemination to the first three cleavages in eggs of *Dendraster excentricus* exposed to ultra-violet light for varying durations at a distance of 30 cm. Each value is the average time-lapse from insemination to the particular cleavage for the reacting half of 12,000 eggs. In the controls the average time-lapse from insemination was 58.2 minutes for first cleavage, 87.8 minutes for second cleavage, and 117.4 minutes for third cleavage.

Duration of exposure (seconds)	Periods when eggs were exposed and time (minutes) from insemination to each of the first three cleavages														
	Before insemination			15 minutes after insemination			30 minutes after insemination			45 minutes after insemination			55 minutes after insemination		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
15	84.3	135.4	178.4	78.7	115.8	166.7	59.3	88.3	133.8	57.4	101.2	127.4	56.8	109.1	180.8
30	85.9	138.3	193.4	94.9	137.2	204.8	61.2	91.9	151.6	57.9	110.9	139.2	55.6	117.9	191.8
45	88.1	168.3	——	95.4	143.5	208.1	62.5	114.1	176.5	59.0	150.5	187.8	57.8	120.8	217.5
60	90.9	174.5	——	99.4	141.8	227.6	65.0	124.7	171.7	59.4	164.8	207.3	56.3	133.5	223.6
75	94.8	——	——	101.6	148.9	——	69.5	117.2	169.8	60.6	173.2	——	57.3	147.4	237.2
90	97.0	——	——	104.8	155.7	——	73.2	121.5	163.4	62.7	177.3	——	56.9	169.7	——
105	——	——	——	108.7	173.4	——	78.8	114.8	168.2	68.7	——	——	58.1	168.3	——
120	——	——	——	111.9	169.7	——	83.1	117.3	174.1	82.3	——	——	58.9	174.8	——

The time-lapse measurements for irradiated eggs were compared with similar measurements for controls and the relation between the measurements is presented as the per cent increase in time-lapse (delay in cleavage time). Graphs of the percentage increase in the time-lapse for cleavage in rayed eggs which reveal the differences in the extent of radiation effect during the various stages in development are shown in Figs. 1 and 2.

In the text figures abscissæ show durations of exposure and ordinates indicate cell division rate as the percentage increase in time-lapse from insemination to cleavage. The groups of curves, Fig. 1*A* and

TABLE III

Average time-lapse (minutes) from insemination to the first three cleavages in eggs of *Urechis caupo* exposed to ultra-violet light for varying durations at a distance of 30 cm. Each value is the average time-lapse from insemination to the particular cleavage for the reacting half of 12,000 eggs. In the controls the average time-lapse from insemination was 70.3 minutes for first cleavage, 101.8 minutes for second cleavage, and 132.5 minutes for third cleavage.

Duration of exposure (seconds)	Periods when eggs were exposed and time (minutes) from insemination to each of the first three cleavages																	
	Before insemination			15 minutes after insemination			30 minutes after insemination			45 minutes after insemination			60 minutes after insemination			70 minutes after insemination		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
15	70.7	107.3	197.4	71.2	109.5	146.3	73.3	107.3	138.3	71.8	105.6	134.6	69.6	102.5	136.7	69.9	98.9	123.6
30	72.8	128.5	238.1	74.8	123.7	197.6	75.4	125.6	168.1	79.2	108.6	148.2	71.0	109.3	148.3	71.1	101.3	132.8
45	—	—	—	77.4	—	—	84.7	—	—	89.7	150.3	—	72.4	113.5	157.2	71.4	109.6	146.4
60	—	—	—	78.7	—	—	98.5	—	—	100.9	—	—	75.6	117.1	169.2	76.8	115.5	158.5
75	—	—	—	—	—	—	—	—	—	—	—	—	82.5	150.3	—	83.2	128.7	172.8
90	—	—	—	—	—	—	—	—	—	—	—	—	91.9	—	—	92.7	—	—

Fig. 2*A*, show the sensitivity of the eggs to ultra-violet radiation when exposures were made at certain stages in development before initial segmentation. The groups of curves, Fig. 1, *B* and *C*, and Fig. 2, *B* and *C*, show the extent to which radiation treatment of the eggs before first cleavage affects the rate of subsequent cell divisions.

Discussion

The interpretation of results on the effects of radiant energy upon living cells is based upon factors which are chiefly of a biological or a

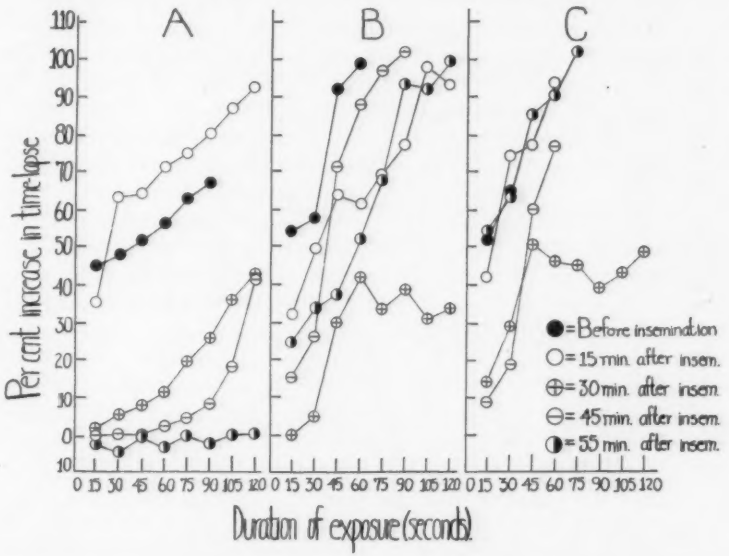

Fig. 1. Curves showing the rate of cleavage in eggs of *Dendraster excentricus* exposed to ultra-violet light at different stages in development. First, second, and third cleavage (*A*, *B*, and *C*).

physico-chemical nature. The significance of the biological aspect of the problem is emphasized by Failla (1924). A factor of importance from this point of view is the fundamental difference in cellular activity, especially in animal ova, during early stages in development.

Since frequent use is made of the rate of first cleavage in dividing eggs as an indicator of radiation effect, it is essential to know the relation between response to exposure and the characteristics of early (pre-cleavage) development. A greater variation occurs in the sequence of events which leads to the initial segmentation of the fer-

tilized egg than is found during the period which precedes any subsequent cleavage in the same egg or during inter-division phases of other dividing cells. Eggs fall in categories with respect to the biological processes which follow insemination and lead to first cleavage, Lillie and Just (1924) and Wilson (1925). The eggs of *Dendraster*

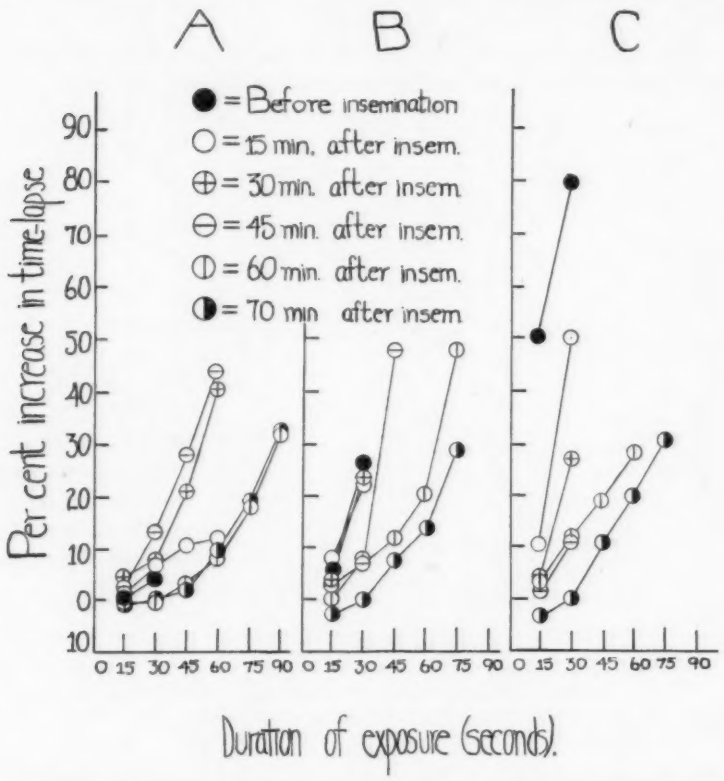

FIG. 2. Curves showing the rate of cleavage in eggs of *Urechis caupo* exposed to ultra-violet light at different stages in development. First, second, and third cleavage (*A*, *B*, and *C*).

excentricus and *Urechis caupo* are so widely separated with regard to internal phenomena during the period of pre-cleavage development that a consideration of the results which have been presented is of interest because of its bearing upon the study of cellular sensitivity to radiation. It is probable that variations in developmental processes,

and the underlying complex of reactions, may be factors in the analysis of radiation effect when the rate of first cleavage in egg cells is the criterion of experimentation.

Comparison of the data in Tables II and III, and of the groups of curves in Fig. 1 with the curves in Fig. 2, reveals a marked difference in the response of egg cells to irradiation during the stages of pre-cleavage development which precede the first cell division. Susceptibility of the eggs to radiation effect (maximum delay in cleavage time) occurs during the period from insemination to the resting nuclear stage. During the mitotic phases which lead to initial segmentation, eggs of the two species are resistant (minimum delay in cleavage time) to the effect of ultra-violet rays. Since results from X-ray studies show a greater effect of exposure during certain mitotic phases,—Richards (1914) for *Planorbis*, found X-rays most effective during the metaphase, —a matter of interest is the fact that no marked susceptibility to ultra-violet radiation was exhibited by eggs of *Dendraster* and *Urechis* during the various phases of the mitotic cycle.

The results cited give evidence of a differential response of fertilized eggs to irradiation which may be correlated with processes involved in pre-cleavage development. Suppression of maturation in certain eggs following exposure to ultra-violet light, Just (1933) for eggs of *Nereis limbata* and Chase (1937) for eggs of *Urechis caupo*, may be a factor in the delay of cleavage in irradiated eggs. Some other factor exists for eggs of *Dendraster*, since the maturation process is not a part of pre-cleavage development. A reasonable probability is the metabolic process which parallels cellular activity during pre-cleavage development. Whitaker (1931) reported a decline in the rate of oxygen consumption during the first 40 minutes after fertilization for eggs of *Nereis limbata* while eggs of sea urchins show an increased rate of metabolism during the first hour after fertilization (see comparisons by Whitaker, 1933). In view of the similarity of earliest development in eggs of *Nereis* and *Urechis* and in eggs of sea urchins and *Dendraster*, it is possible that approximately equivalent rates of metabolism are concomitant with developmental processes which are suppressed or inhibited by ultra-violet radiation. Alteration of metabolic rate conceivably may cause acceleration or retardation of phases of cellular activity which underlie cell division, and thus affect segmentation tempo.

Another agency which may affect the rate of cell division in developing eggs is the change in the consistency of egg protoplasm following exposure to radiation. During the experiments eggs of both species were overexposed (rayed for durations of four to seven minutes) at

different stages in development. When the eggs were observed about 20 minutes after irradiation a very definite flowing of the endoplasm was seen. Streaming of the inner protoplasm of the eggs was not observed for any of the durations of exposure which are reported in the data, but it is possible that an unobservable change in viscosity may have followed some of the longer irradiations. Measurements of the changes in the viscosity of *Arbacia* egg protoplasm following exposure to ultra-violet light are presented by Heilbrunn and Young (1930). According to the changes in the viscosity of egg protoplasm it is reasonable to assume that reactions may be affected which influence the rate of cleavage.

The delay in segmentation time of the second and the third cleavages (see Fig. 1, *B* and *C*, and Fig. 2, *B* and *C*) occurs as a result of the irradiation of the egg during periods of development before first cleavage. Two explanations of the effect are: (*a*) initial injury of the egg caused by the pre-cleavage exposures; (*b*) a latent effect of radiation which varies in extent for each of the two cell divisions with respect to the stage in development when exposures are made. In both cases failure of recovery from the effect of radiation is indicated, and the effect upon the rate of cleavage is apparently a function of the duration of exposure. The theory that the eggs are injured in some way is the more tenable, although there is a strong evidence of a latent effect of radiation, especially in eggs of *Dendraster excentricus*, which were rayed just before the first cleavage.

There is need of further studies on the effect of radiation upon developing eggs which, from a purely biological point of view, may serve for more adequate analysis of radiation effects upon living cells. Work is now in progress on the percentage of cleavage in developing eggs as an indicator of susceptibility or resistance to irradiation.

Summary and Conclusions

1. The effect of ultra-violet light upon the rate of the first three cell divisions in eggs of *Dendraster excentricus* and *Urechis caupo* is an increase in segmentation tempo which is apparently a function of duration of exposure. This delay in cleavage time occurs regardless of the period in development from insemination to first cleavage when exposures are made.

2. The sensitivity of fertilized eggs to radiation effect is dependent upon the stage in pre-cleavage development when exposures are made. Since developmental processes vary for eggs of different species, during the period from insemination to first cleavage, differences in the degree of sensitivity may be expected.

3. Evidence for a differential response of fertilized eggs to irradiation is presented which may be correlated with radiation effect upon a process of pre-cleavage development, or upon underlying reactions which are concomitant with phases of cellular activity which lead to cell division.

4. The viability of fertilized eggs of the two species varies over the range of exposures, longer durations preventing cleavage in eggs of *Urechis*. In view of this fact, adequate measurements of segmentation rate may not be recorded at all exposures to ultra-violet light.

5. The analysis of radiation effect upon living cells, from a purely biological point of view, should be based upon conclusive data which take into consideration the vast differences in cellular activity during pre-cleavage and inter-division stages. In this connection, it is suggested that a more appropriate criterion for cellular sensitivity to radiation effect may be the determination of the percentage of cleavage (survival) in egg cells of the sand dollar-sea urchin type of developmental activity.

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THE MORPHOLOGY AND LIFE CYCLE OF THE TREMATODE HIMASTHLA QUISSETENSIS (MILLER AND NORTHUP, 1926)

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The trematodes of the family Echinostomatidæ comprise a large number of genera which infest the alimentary tract of birds and mammals. The family and several genera were characterized by Dietz (1910). In a preliminary paper, Dietz (1909) had erected the genus *Himasthla*, with *H. rhigedana* as the type species, and in it he included *H. alincia* Dietz, *H. leptosoma* (Creplin), *H. militaris* (Rudolphi), *H. elongata* (Mehlis), and *H. secunda* (Nicoll). Linton (1928) described specimens which he identified as *H. elongata* (Mehlis), and others which he named *H. incisa* new species. A new human parasite, *H. muehlensi*, was described by Vogel (1933), who included a tabular description of all known species of *Himasthla*. Palombi (1934) described the metacercaria of a new species, *H. ambigua*, from the gills of *Tapes decussatus*.

The first complete life history of an echinostome to be recorded was that of *Echinostoma revolutum* by Johnson (1920). Subsequent life cycles, demonstrated experimentally, include that of *Hypoderæum conoideum* (Bloch) by Mathias (1925), of *Echinoparyphium recurvatum* (von Linstow) by Mathias (1927), of *Echinoparyphium aconiatum* Dietz by Riech (1927), of *Euparyphium murinum* Tubangui by Tubangui (1932), of *Euparyphium ilocanum* (Garrison) by Tubangui and Pasco (1933), of *Euparyphium malayanum* by Rao (1933), of *Echinoparyphium recurvatum* by Rašín (1933), of *Nephrostomum ramosum* by Azim (1934), of *Echinostoma coalitum* by Krull (1935), and of *Echinostoma revolutum* by Beaver (1937). The monograph of Beaver contains a review and analysis of the earlier papers. He noted certain discrepancies in the account of Johnson (1920) and suggested that the observations were made on material of more than one species. Indeed, the older descriptions are so incomplete and so inaccurate that it is exceedingly difficult to determine how many times one species has been redescribed or how many species have been confused. This condition is particularly prominent and perplexing when both adult and larval stages are considered together. Referring to descriptions of a

group of eleven species of echinostome cercariæ, Beaver, p. 17, stated, "While it is not certain that they all are identical, it is certain that they can not be distinguished from each other." He pointed out that *Echinostoma revolutum* is cosmopolitan in geographic distribution and has little specificity in any of its parasitic stages. Using biometric methods on specimens reared under known, controlled conditions, he measured the normal variation in this species, noting especially the modifications induced in genetically similar material as a result of development in different avian and mammalian hosts. In the light of the more complete and precise knowledge concerning *E. revolutum*, Beaver made a critical examination of the descriptions of related species. Eight of them were reduced to synonymy and eight others were listed as of doubtful standing.

Other important contributions to the life history of these parasites include those of Ciurea (1920) and the reports of several Japanese investigators whose accounts, published in Japanese, are unfortunately not readily available. In the life cycles described, the cercariæ have been found to encyst in various mollusks, fishes and tadpoles.

Although the life history has not been completely demonstrated previously for any other species of *Himasthla*, there have been certain important and valuable contributions. Villot (1879) redescribed *H. leptosoma* (Creplin) and identified an encysted metacercaria from *Scrobicularia tenuis* as a stage in the life cycle of the worm. He traced the successive stages of development from the encysted metacercaria to the mature adult in the intestine of *Tringa variabilis*. Nicoll (1906*a*, 1906*b*) showed the close morphological agreement between an echinostome metacercaria, which he found encysted in *Cardium edule*, *Mytilus edulis* and *Mactra stultorum*, and an adult which he described as *Echinostomum secundum*. Lebour (1908) confirmed the account of Nicoll and reported an echinostome cercaria with twenty-nine cephalic spines which she believed to be the larva of *E. secundum*. Experimental infection under controlled conditions was not secured.

The life cycle of an echinostome cercaria which occurs in *Nassa obsoleta* at Woods Hole, Massachusetts, has been experimentally traced and a preliminary note was published, Stunkard (1934*a*). A more extended account was presented (Stunkard, 1937). There is adequate evidence that the cercaria is identical with the one described by Miller and Northup (1926) as *Cercaria quissetensis* and the adult is specifically identical with certain of the worms identified by Linton (1928) as *Himasthla elongata*. Since the sexually mature specimens are specifically distinct from *H. elongata*, the new combination *Himasthla quissetensis* was adopted.

To be convincing, life history studies must be carried on under well controlled conditions. The hosts used for experimental infections must be free from a previous infection which could be confused with the experimental one, or the course of development of the parasite must be followed at such short intervals that the organism can be identified at every stage. In the present study, the gulls and terns used as final hosts were removed from the nesting grounds as soon as they were hatched and, since they had never been fed by their parents, were known to be free from infection. They were fed on fish which contained no trematode larvæ, so the experimental infection was not complicated. Rats used in the experiments were laboratory raised and harbored no trematode parasites. The cercariæ of *H. quissetensis* were observed to penetrate into the gills and other organs of mollusks and encyst there. These encysted metacercariæ were fed to rats, terns and gulls, all of which were known to be free of trematode infection. The metacercariæ are infective for the final host soon after encystment and undergo little development in their cysts. It appears, therefore, that the cyst serves as a protective device to carry the larva through the acid digestion of the stomach, and that the metacercaria emerges in the small intestine of the bird or mammal. Successive stages of development were recovered from the intestine of experimentally infected birds.

The Sexual Stage in the Life Cycle

The Adult

About two hundred worms (Fig. 8) were recovered from the intestine of a herring gull, *Larus argentatus*, thirty-one days after the ingestion of experimentally infected mollusks. The shape of the body is portrayed in the figure. It is capable of much elongation and contraction and the appearance of the worms and their internal organs is modified accordingly. All of the specimens contain eggs and although the terminal portion of the uterus is usually empty, many eggs were being passed in the feces of the bird. It is possible that the region of the body immediately behind the cirrus sac would have been slightly wider if the worms had been older or more completely matured. When alive and greatly extended the sides are smooth but in all well-fixed specimens the contraction of circular and longitudinal muscles gives the edges of the body a crenated or ringed appearance. Fixed and stained specimens measure from 5 to 10.2 mm. in length and 0.5 to 0.75 mm. in greatest width. The anterior end bears a reniform collar, interrupted ventrally, and at this level the body measures about 0.3 mm. in diameter. On the collar there are thirty-one cephalic spines,

arranged in a single row except for the lateral corners where two spines are situated between and behind the others. The spines measure from 0.045 to 0.058 mm. in length and 0.014 to 0.020 mm. in width. The corner spines are only slightly shorter than those in the row. The size and arrangement of the cephalic spines agree with the description and figure of these structures on the worms identified as *H. elongata* by Linton (1928). In the preacetabular region the cuticula is beset with flattened, scalelike spines which are largest near the anterior end and become progressively smaller posteriorly. The acetabulum measures 0.2 to 0.4 mm. in diameter and it is situated about the same distance behind the oral sucker. In a specimen 7.8 mm. long, the acetabulum is 0.36 mm. in diameter.

Digestive System.—The oral sucker is subterminal, spherical to ovoid in shape, and measures from 0.07 to 0.125 mm. in length by 0.1 to 0.135 mm. in width. The prepharynx is short and the pharynx measures from 0.1 to 0.13 mm. in length by 0.06 to 0.09 mm. in width. The esophagus extends to the level of the anterior margin of the acetabulum and the ceca to the posterior region of the body.

Male Genital System.—The testes are elongate, faintly lobed structures, situated in the caudal third of the body. The anterior testis measures 0.5 to 1 mm. in length and 0.25 to 0.33 mm. in width. The posterior testis is usually somewhat longer and measures from 0.7 to 1.1 mm. in length and 0.2 to 0.26 mm. in width. The testes are close together in small or contracted specimens but in larger or extended ones they are separated by a distinct interval. The sperm ducts are very small and could not be observed in whole mounts. They were traced in one series of sections. A vas deferens arises from the anterior, ventral surface of each testis and passes forward, median and ventral to the cecum, the duct from the anterior testis on the left and that from the posterior testis on the right side of the body. Both open into the caudal end of the cirrus sac where they discharge into a large, coiled, seminal vesicle which occupies the posterior third to half of the cirrus sac. The cirrus sac is long and the postacetabular portion winds about on the dorsal side of the body. Its extent is indicated in the figure but measurements of length in an organ of this shape are not significant. The cirrus is armed with small recurved spines. The genital pore is median, at the anterior border of the acetabulum.

Female Genital System.—The ovary is spherical to oval, usually broader than long, 0.1 to 0.22 mm. in diameter. It is situated near the median line, a short distance in front of the cephalic testis. The oviduct arises at the caudal end and passes backward to the oötype which is surrounded by the cells of Mehlis' gland. From the oötype,

Laurer's canal proceeds in a sinuous course to the dorsal surface. The vitellaria are lateral to the intestinal ceca and extend from a level slightly in front of the caudal end of the cirrus sac to the posterior end of the body. In the fields on either side of the testes, ordinarily there are no vitelline follicles and a duct connects the separated portions of the glands. In one specimen a few small follicles were present on one side of the posterior testis. There is a cluster of follicles on either side at the level between the testes. Immediately in front of the cephalic testis, ducts arise from the ventral sides of the vitellaria and increase in size as they pass dorsally and medially where they unite to form a short common duct which discharges into the oötype. There is no seminal receptacle, but the initial portion of the uterus is filled with spermatozoa. The uterus extends in a sinuous course, backward to the level of the cephalic testis and then forward to the metraterm which lies below the caudal end of the cirrus sac. Both metraterm and cirrus sac pass on the dorsal side of the acetabulum. The eggs are large, oval, operculate, thin-shelled, and measure from 0.1 to 0.125 mm. in length by 0.06 to 0.08 mm. in width. They do not develop in the worm and those in the terminal part of the uterus contained only a fertilized ovum and masses of vitelline cells. Indeed, the terminal half of the uterus contains very few eggs, and it appears that they are passed rapidly through this portion of the organ.

The Asexual Stages

The cercariæ develop in rediæ which occupy the interlobular lymph spaces in the digestive gland of *Nassa obsoleta*. Experimental infection of the snail was not secured as the work was done during the summer at the Marine Biological Laboratory, Woods Hole, Massachusetts, and development of the eggs was so slow that hatching was not obtained before the end of the season. Johnson (1920) and Beaver (1937) reported that eggs of *Echinostoma revolutum* hatch in three to four weeks after they are passed in the feces. In *Euparyphium ilocanum*, according to Tubangui and Pasco (1933), the eggs require from two to five or six weeks to complete their development. Rašín (1933) found that eggs of *Echinoparyphium recurvatum* require two weeks at 25° and three weeks at 20° for the development of the miracidium. In all echinostome species in which development has been studied, the eggs contain unsegmented ova when they are passed. From two to five weeks, depending on the temperature, are required for development of the miracidium which then emerges from the shell and penetrates into the snail which serves as the first intermediate host. The development of all members of the family Echinostomatidæ undoubtedly follows the

same general plan and accordingly it is probable that the miracidia of *H. quissetensis* complete their development in the eggs, emerge and penetrate into *Nassa obsoleta*. For *E. revolutum*, Johnson (1920) postulated that the mother rediæ were derived by metamorphosis from miracidia. Later studies on other echinostomes, however, indicate that in this family as in other digenetic trematodes, the miracidium metamorphoses into a sporocyst and the next generation is produced in the sporocyst. It is, of course, possible that there may be only a single redia in the sporocyst as reported by Linton (1914) for *Parorchis avitus* and by Stunkard (1934*b*) for *Typhlocælum cymbium*. In the latter cases, however, the mother redia is well developed in the miracidium.

In natural infections of *Nassa obsoleta*, the interlobular lymph spaces in the digestive gland are filled with developing rediæ and cercariæ. Often the gland is much atrophied and the parasites are present in enormous numbers. In a heavy infection there may be thousands of large rediæ, filled with developing cercariæ. In addition there may be hundreds of small rediæ, often not more than one-half the size of a mature cercaria, free in the tissues of the snail. Their number is too large to believe that they have been formed in a primary sporocyst and, since they are of different sizes, they must have been produced more or less continuously. Thousands of rediæ have been taken from crushed snails and dissected under a binocular microscope but none was ever found in which young rediæ could be positively identified. It is impossible to determine whether the germinal masses in a redia are developing rediæ or cercariæ, and in all cases where development had proceeded to such a stage that positive identification could be made, the larvæ were cercariæ. The relative numbers of rediæ of different sizes indicated strongly the existence of two generations of rediæ but dissections gave no certain evidence of mother rediæ.

In order to discover the source of the small rediæ, a well-infected snail was cut in serial sections. Large numbers of small rediæ were found in the tissues and others were found (Fig. 1) within the body of mother rediæ. The identification is unmistakable. The pharynx of a redia can be distinguished from the oral sucker of a cercaria by the number and arrangement of the nuclei. The feet of the daughter redia are clearly visible, and there is no oral sucker or acetabulum. An intestine is present, although empty and collapsed. Behind the intestine there is a cleft which contains germinal cells and small germ balls, young stages of the next or cercarial generation. In addition to a daughter redia, each mother redia contained from six to sixteen other germinal masses but they were undifferentiated and their appearance

gave no indication concerning their nature. It would be impossible to determine whether they were young rediæ or cercariæ if it were not for the presence of the daughter redia in the same mother redia. This discovery explains the difficulty concerning the source of the small rediæ in the tissues. The daughter rediæ are produced singly and emerge from their parental generation at a relatively early stage, before they can be distinguished readily and with certainty from developing cercariæ. There is no evidence to suggest that both rediæ and cercariæ are produced in the same redia.

The Redial Generations

In early stages mother and daughter rediæ do not differ essentially from each other in size, shape, or general appearance. The two generations can be distinguished only by their progeny and by the changes which the development of the filial generation produces in them. The mother rediæ produce daughter rediæ which mature singly, whereas the daughter rediæ produce cercariæ which mature in numbers, and when filled with cercariæ the daughter redia is much distended. Mature mother rediæ (Fig. 1) in the tissue of the snail measure from 0.5 to 0.7 mm. in length and 0.14 to 0.2 mm. in width. The pharynx measures 0.05 to 0.06 mm. in diameter.

The smallest daughter redia which was sufficiently developed to be recognized is the one found in a mother redia (Fig. 1). It measures 0.13 mm. in length and 0.056 mm. in width. The pharynx is 0.033 mm. in diameter. There are both anterior and posterior locomotor appendages or "feet." Older daughter rediæ (Fig. 2), which have emerged from their parental generations and are actively migrating in the tissues of the snail, measure from 0.2 to 0.3 mm. in length and 0.1 to 0.14 mm. in width. At this stage the pharynx measures approximately 0.04 mm. in diameter, the intestine is filled with food material and extends to the level of the posterior locomotor appendages. Around the posterior part of the intestine there is a body cavity which contains germinal cells. As the rediæ mature, the body wall becomes filled with orange pigment, the intestine and anterior locomotor appendages remain small, while the caudal region of the body and posterior locomotor appendages increase in size. The body cavity enlarges, becomes filled with cercariæ in various stages of development (Fig. 3), and the birth pore becomes functional. The redia is motile and the body may assume different shapes. It may contract to a short, cylindrical form in which the posterior locomotor appendages are hardly visible, or it may extend to a long vermiform shape in which the caudal end and posterior locomotor appendages form a tripodal

support for the forward protrusion of the body. The anterior end is especially mobile. In front of the pharynx there is an active oral funnel or collar. It may form a small protuberance, about one-half as wide as the rest of the body, which disappears on retraction. Mature daughter rediæ (Fig. 3) measure from 0.8 to 1.6 mm. in length and from 0.1 to 0.25 mm. in width. The pharynx is 0.05 to 0.065 mm. in diameter.

The Cercaria

During the past seven seasons over ten thousand specimens of *Nassa obsoleta* have been examined for larval trematodes. They were isolated for forty-eight hours to find the specimens from which cercariæ were emerging. About 1 per cent of the snails was infected with *Cercaria quissetensis* although cercariæ emerged spontaneously from only about one-half of these specimens. The other infections were discovered on crushing and examining the snails. Four snails were infected with both *C. quissetensis* and *C. lintoni*.

After emergence from the snail the cercaria of *Himasthla quissetensis* swims actively for six to twelve hours. In swimming, the body is bent ventrally until it is almost spherical; the tail is extended and lashes vigorously. The swimming movement causes the cercariæ to rise in the water and during the period of active swimming they are uniformly distributed throughout a large container. Occasionally, for a few seconds the tail may cease to beat while the larva elongates and manifests a serpentine movement. When the tail is quiet, the larva slowly sinks. After six to ten hours, the larvæ do not rise high in the water

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Abbreviations

ac acetabulum
al anterior locomotor appendage
cd excretory collecting duct
cs cirrus sac
dr daughter redia
gm germinal mass
in intestine
os oral sucker
ov ovary
ph pharynx
pl posterior locomotor appendage
ts testis
ut uterus
vt vitellaria

FIG. 1. Section of mother redia, showing daughter redia and germinal masses The daughter redia is 0.13 mm. long.

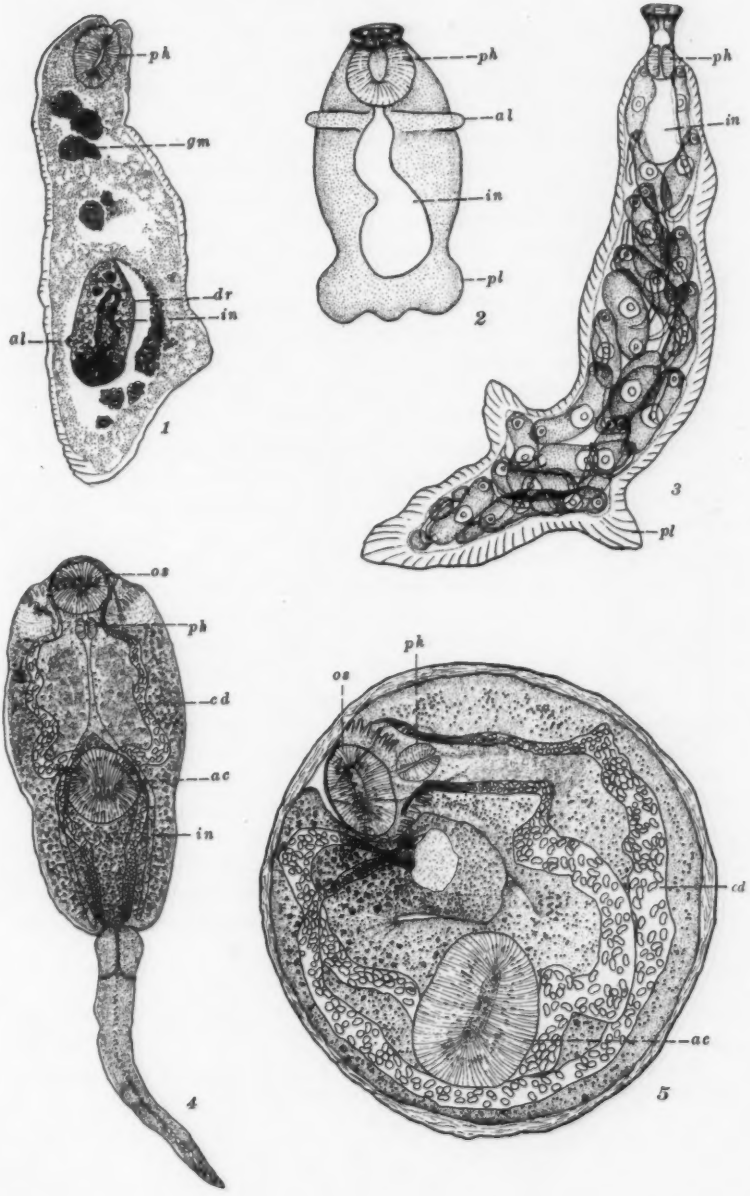
FIG. 2. Daughter redia after emergence from mother redia; drawn from a living specimen. Daughter redia is 0.2 mm. long.

FIG. 3. Mature daughter redia filled with cercariæ; drawn from a living specimen

FIG. 4. Cercaria; drawn from a fixed and stained specimen.

FIG. 5. Metacercaria; drawn from a living specimen.

PLATE I



and either swim near the bottom or creep by use of the suckers. When creeping, the tail is quiet. A report on the behavior of the cercariæ in normal and dilute sea water was made by Stunkard and Shaw (1931). After twenty-four hours in sea water there is a tendency for the cercariæ to encyst. If they do not encyst, they die in about thirty-six hours.

Fully developed, naturally emerged cercariæ (Fig. 4) are elongate oval in outline. When extended the body is very narrow and when contracted the anterior end is much widened. The body measures from 0.28 to 0.65 mm. in length and 0.06 to 0.15 mm. in width. When the larva is extended, the oral sucker is protruded, and when retracted, the anterior end of the body is flattened. On the anterior portion of the body and on the tail there are small papillæ, each of which bears a delicate, bristle-like process. The base of the tail is slightly subterminal and may be either wide or narrower than the posterior tip of the body. Contracted, the tail measures about 0.17 mm. in length and 0.048 mm. in width at the base. It is almost round in cross-section and gradually tapers to the tip. In swimming, the tail extends two to three times its length when contracted and lashes so violently that when the body is attached firmly, it may tear itself loose. The anterior end of the body is thickened to form a collar which bears the heavy spines characteristic of the species. The spines can be seen only under favorable optical conditions and it is very difficult to count them. The acetabulum is situated behind the middle of the body and measures from 0.07 to 0.085 mm. in diameter. Under slight pressure it may be as large as 0.084 to 0.091 mm. in diameter.

The digestive system may be demonstrated clearly by the use of neutral red. The oral sucker is 0.04 to 0.045 mm. in diameter. The pharynx is spherical to oval, about 0.018 mm. in diameter, situated approximately the same distance behind the oral sucker. The esophagus extends almost to the level of the acetabulum and the ceca terminate near the posterior end of the body.

Unicellular glands are abundant throughout the body. In the region between the suckers there are many penetration glands with granular cytoplasm. They are lobed and their number could not be

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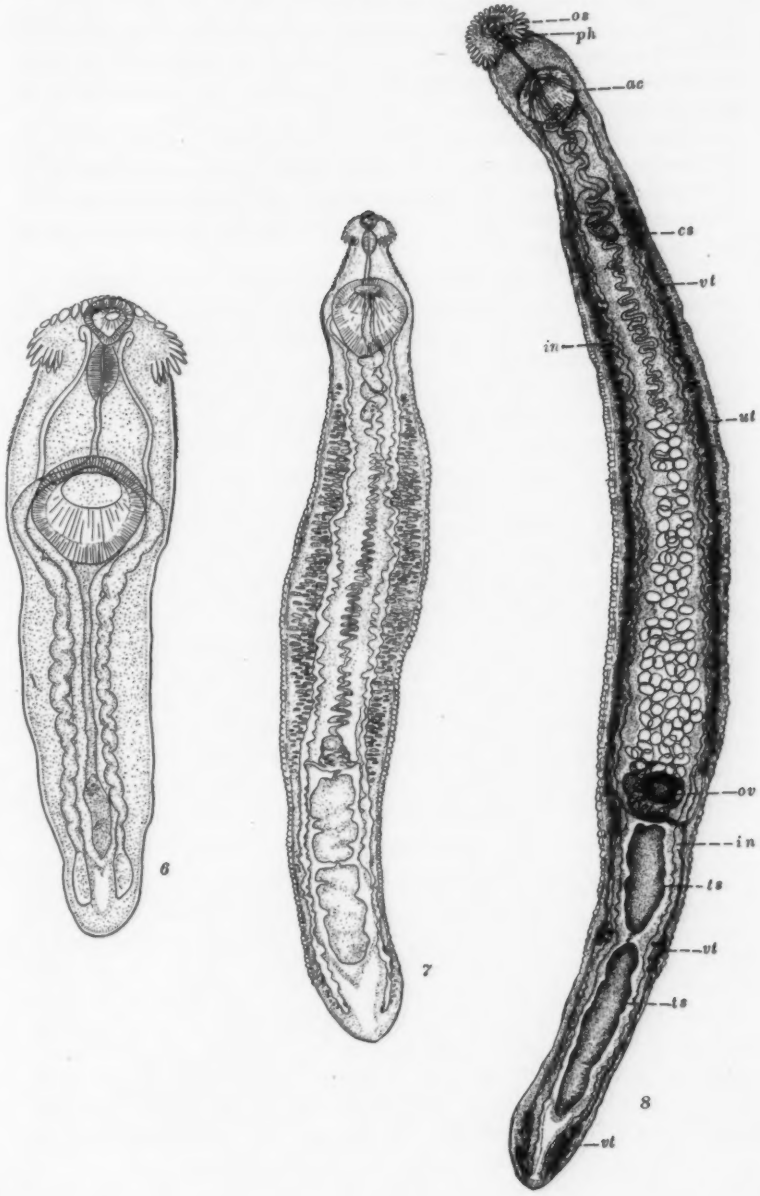
FIG. 6. Immature worm, 3 days in tern; drawn from fixed and stained specimen.

FIG. 7. Immature worm, 12 days in gull; drawn from fixed and stained specimen.

FIG. 8. Adult worm from the intestine of *Larus argentatus*, thirty-one days after cyst was fed; drawn from a fixed and stained specimen.

PLATE II



determined. They do not stain differentially with neutral red. Ducts pass forward on the dorsal side of the body to open at the anterior tip. Much of the body surface on the dorsal side is underlaid with cystogenous glands, in which the secretion occurs in the form of bacilliform rods. Ventrally there are glands which stain intensely with eosin and erythrosin.

The genital anlage consists of a cluster of deeply-staining cells near the caudal end of the body and a row of cells which extends forward to a second cluster near the anterior margin of the acetabulum. The posterior group is the rudiment of the gonads and the anterior one of the copulatory organs.

The excretory system is difficult to trace, because in mature cercariæ the body is so filled with glandular cells, and in immature ones the tissues are so fragile that they will not stand the amount of pressure necessary to demonstrate the tubules and flame cells. There are two excretory pores, one on either side, near the proximal end of the tail. The ducts from the pores unite in the base of the tail and open into a small vesicular bladder in the caudal end of the body. From the bladder, collecting ducts pass forward on each side. These ducts are median to the ceca. They bend mediad in front of the acetabulum and then cross, ventral to the intestinal ceca, to the lateral sides of the body where they continue anteriad to the level of the oral sucker. The collecting ducts contain concretions; those near the ends of the ducts are small, 0.001 to 0.002 mm. in diameter, while those in the region between the suckers are large and measure about 0.005 by 0.009 mm. Near the anterior end of the body the collecting ducts become continuous with the recurrent excretory tubules which pass backward almost to the posterior end of the body. At their caudal ends the recurrent tubules divide to form the secondary and tertiary tubules which lead to the flame cells. The exact arrangement of these tubules and number of flame cells were not determined.

The Metacercaria

Occasionally a cercaria which has been swimming for several hours may encyst in a container or on a slide. Encystment may occur in the presence of irritating substances, e.g., vital stains, which were used to study the penetration and cystogenous glands. Since the cercariæ do not ordinarily encyst in sea water, they were placed in dishes with various animals to discover the intermediate host or hosts in which they pass the next stage of their existence. A cercaria was observed to penetrate into the gill of *Mya arenaria*. It required about forty minutes to enter and encyst. In the process of penetration and encystment, the glands of the cercaria are emptied and a thin-walled cyst is

formed. A specimen of *M. arenaria* was placed in a dish with many swimming cercariæ. On dissection of the mollusk four hours later, five cercariæ had lost their tails and were penetrating, one was found in the process of encysting, and twelve metacercariæ were recovered. Penetration and encystment was obtained in the mantle, gills, and foot of *Mya arenaria*, *Modiolus modiolus*, *Mytilus edulis*, *Cumingia tellinoides*, *Pecten irradians*, *Ensis directus*, and *Crepidula fornicata*. Cysts containing metacercariæ (Fig. 5) are spherical to oval, depending on relative pressure at the place of encystment, and measure from 0.14 to 0.19 mm. in diameter.

Development in the Final Host

Experimentally infected specimens of *M. arenaria* were fed to two white rats, three terns, *Sterna hirundo*, and five herring gulls, *Larus argentatus*. The rats did not pass eggs in the feces and both were negative when dissected three and eight weeks, respectively, after feeding. It appears, therefore, that the rat is not a suitable host for the development of the worms. The terns were killed, one day, three days, and twenty days after feeding. The metacercariæ excysted in the intestine of the terns and young worms were recovered from the first two birds examined. The third tern did not pass eggs in the feces and was negative when dissected twenty days after feeding. The gulls were killed, two, three, twelve, thirty-one, and forty-five days after feeding. Large numbers of worms in different stages of development were recovered from the first three gulls, about two hundred gravid worms were found in the fourth gull, and a single worm was present in the intestine of the last one dissected. A young worm removed after one day in the tern measured 0.6 mm. long and 0.154 mm. wide. The spines on the collar were 0.025 mm. in length; the acetabulum measured 0.1 mm. in diameter. The oral sucker was 0.056 mm. in diameter and the pharynx 0.04 mm. long by 0.028 mm. wide. A specimen (Fig. 6), recovered after three days' development in the gull, measured 0.74 mm. in length and 0.23 mm. in width. The collar spines measured 0.0294 mm. in length and 0.0077 mm. in width at the base; the acetabulum measured 0.14 mm. in diameter. The oral sucker was 0.06 mm. in diameter and the pharynx 0.04 mm. long by 0.035 mm. wide. Specimens (Fig. 7), recovered twelve days after they were fed to the gull, measure from 3 to 4 mm. in length. Although they are only about one-half grown, most of them contain eggs in the uterus. These studies have shown that the metacercariæ do not undergo development in the cyst and that the mollusk is actually little more than a transfer host. The metacercariæ were infective for birds

three days after encystment and developed to maturity only in the intestine of *Larus argentatus*.

Discussion

Miller and Northup (1926) examined 8,875 specimens of *Nassa obsoleta* from Quamquisset Harbor and found only a single species of echinostome cercaria which they described and named *Cercaria quissetensis*. In the present study over 10,000 specimens of *N. obsoleta* have been examined. All came from the Woods Hole region and about 1,000 of them from the same harbor where Miller and Northup obtained their material. A single species of echinostome cercaria was found which is so similar to that described by Miller and Northup that the two must be regarded as identical. The measurements agree closely and the differences between the descriptions are readily explainable. Miller and Northup reported only 27 cephalic spines. In the present study the exact number, 31, was first counted in the metacercaria and later confirmed in the cercaria.

In the preliminary papers (Stunkard 1934*a*, 1937), the adult worms recovered from the herring gull were regarded as specifically identical with those described by Linton (1928) from four species of Larus and from *Nycticorax nycticorax* and identified by him as *Himasthla elongata* (Mehlis). Although he referred his specimens to *H. elongata*, Linton noted certain differences between them and previous descriptions of that species. Linton's determination was questioned by Vogel (1933) and comparison of the present worms with the description of *Himasthla elongata* as given by Dietz (1910) shows such marked differences that they cannot be included in the same species. There are constant differences in the number and arrangement of the cephalic spines, size of organs, shape of testes, and in distribution of the vitellaria. In *H. elongata* the vitelline follicles are continuous, whereas in the species which I have studied these glands are interrupted at the levels of the testes. Since this species is distinct from *H. elongata*, the name given by Miller and Northup to its cercarial stage was adopted. The specimens identified as *H. elongata* by Linton were accordingly referred to *H. quissetensis*.

In the paper cited, Linton (1928) reported on several hundred specimens of *Himasthla* which had been collected at Woods Hole, Massachusetts, from four species of *Larus* and from *N. nycticorax*. He stated, "These distomes, while they vary considerably in size and proportions, appear to belong to the same species, and are in such close agreement with *H. elongata* that it seems best to refer them to that species in spite of the difference in the number of circum-oral spines.

H. elongata is characterized by having 29 circum-oral spines, of which the two which are situated at each angle of the oral disc are smaller than the others. In all of the specimens in which they could be distinctly seen in the Woods Hole material, the number of oral spines was found to be 31, arranged as shown in Figure 18." In this figure, 27 spines are situated in a single row with two between and behind the others at the ends of the row.

In addition to the specimens referred to *H. elongata*, Linton described two specimens from the white-winged scoter, *Oidemia deglandi*, as a new species which he named *Himasthla incisa*. In this species he reported that there are about 27 cephalic spines. The type specimen of *H. incisa* and representative specimens of the worms referred to *H. elongata* were deposited in the United States National Museum.

Lack of agreement between the number of cephalic spines on specimens referred by Linton to *H. elongata* and the number previously reported for that species, together with differences in the arrangement of the vitellaria in Linton's specimens and those of *H. quissetensis* have created a problem of specific determination. For comparison with material of *H. quissetensis*, Linton's specimens were borrowed from the U. S. National Museum. There were five slides, numbered 7921 to 7925 inclusive. The type specimen of *H. incisa* was mounted on slide No. 7925. This worm has 31 cephalic spines but the anterior part of the specimen is twisted and flattened, so their arrangement at the ends of the row is not symmetrical. The worm has heavy muscles and the cuticula bears prominent spines which extend posteriad as far as the level of the testes. The vitellaria are massive and extend along the sides of the body without interruption. Other specific features are presented in the measurements and figure of Linton. The specimen is clearly distinct from *H. quissetensis* and from all other described species. All of the other specimens had been identified as *H. elongata* by Linton. A single specimen from *Larus marinus* was mounted on slide 7923. It has 31 cephalic spines and the vitellaria are interrupted at the testicular zones. In both of these features as well as in total size and in the position and size of the individual organs it agrees with *H. quissetensis*. Consequently, I believe that this specimen should be assigned to that species. Nine specimens from *Larus argentatus*, which obviously belong to the same species, are mounted on slide 7921. They agree with Linton's figure 17 and all of them have 29 cephalic spines, 25 arranged in a single row with two between and behind the others at the tips of the collar. It is apparent that these specimens are distinct from the two described previously and their systematic position is doubtful. They may belong to *H. elongata* as Linton believed. A

single specimen from *Nycticorax nycticorax* is mounted on slide 7924. It bears 29 cephalic spines and is probably identical with the worms from *L. argentatus* on slide 7921. Three specimens from *Larus delawarensis* are mounted on slide 7922. They have 29 cephalic spines, and although they are immature, the worms agree structurally with those on slides 7921 and 7924.

Subsequently, Professor Linton kindly sent additional material from his own collection. In a personal communication (April 11, 1938) he wrote, "Upon looking over my slides I find that I must have included a lot from the Herring Gull in my former report that were not *H. elongata*, as I find only 11, instead of 34 slides of that species." Among the specimens received from Professor Linton there is one which has 31 cephalic spines and interrupted vitellaria and which I regard as *H. quissetensis*. The others have 29 cephalic spines and are identical with the specimens on slides 7921 and 7924 from the U. S. National Museum collection.

It appears, therefore, that the specimens identified by Linton as *H. elongata* belong to two distinct species. Most of the worms agree with his figure 17 and they may be *H. elongata*. They have 29 cephalic spines and the measurements of twelve representative specimens are intermediate between or overlap the figures given by Dietz (1910) as characteristic for *H. elongata* and *H. militaris*. In my opinion the worms might with equal justification be referred to either of the two species. On the other hand, they may belong to neither. The second group of specimens referred by Linton to *H. elongata* have 31 cephalic spines and agree with the worms which I have described as *H. quissetensis*. The anterior end and cephalic spines of one of these specimens is shown in Linton's figure 18.

The precise status of the present and other species which have been referred to the genus *Himasthla* is doubtful. Until the amount of variation which naturally occurs in a species is determined, and its limits defined, final specific determination is impossible. In a related species, *Echinostoma revolutum*, Beaver (1937) has studied the amount and nature of variation. Certain of his observations are pertinent to the problem at hand. He found, p. 19, "The range then for mature worms is from 4 to 30 mm. in length. This size range is so great that a description of any one size is grossly inadequate for an accurate diagnosis of all other sizes, and a description by the ordinary methods is impractical when applied to worms having so great a range in size. This would not be true, however, if it were possible to give proportionate measurements applicable to all sizes which, as shown below, cannot be done. Practically every feature has been measured and

plotted; and in no instance is the proportion between two organs or structures a constant throughout the series." He stated, p. 26, "Egg size varies with the age of the worm and is possibly somewhat altered by the host. The younger worms produce eggs with a very great range in size, and the average length is much greater than in more mature ones. The range in size for the eggs of the worms from all hosts was found to be 91 to 145 microns by 66 to 83 microns." Among other conclusions he reported, p. 65, "It is shown by this study that the cephalic spination is the most reliable character for diagnosis of the adult worm."

Sprehn (1932) listed *H. militaris* (Rudolphi, 1803) and *H. secunda* (Nicoll, 1906) as synonyms of *H. leptosoma* (Creplin, 1829) and Palombi (1934) admitted the probable identity of *H. leptosoma* and *H. secunda*. Since three of the four species of *Himasthla* which bear 29 cephalic spines were regarded as identical by Sprehn, one is led to question why the other one, *H. elongata* (Mehlis, 1831) was accepted as valid. There appears to be no better reason for retaining *H. elongata* than the other specific names which were dropped in synonymy. If the opinion of Sprehn is correct, the name of the species is *H. militaris* and not *H. leptosoma*.

With reference to *H. quissetensis*, it is not impossible that this species is identical with *H. alincia* Dietz, 1909, which also has 31 cephalic spines. The description of the latter species is based on a single specimen from *Tringa cinclus*, collected in Brazil. The figure of Dietz suggests the appearance of a worm which was dead before fixation and consequently much elongated. The worm is much more extended than the specimens which I have studied but the only obvious morphological difference between them is in the anterior limits of the vitellaria, which in *H. alincia* do not extend to the level of the cirrus sac.

A further case of specific identity is suggested between *H. quissetensis* and *H. muehlensi*, the species described by Vogel (1933) from man. A comparison of the specimens at hand with the description of *H. muehlensi* shows much similarity especially in the distribution of the vitellaria. The worms studied by Vogel were slightly larger and apparently contained one more spine in the cephalic coronet. But those specimens were dead and somewhat macerated; in none of them was the crown of spines complete and intact, and the absence of lateral crenations in the body wall may be correlated with the greater length of the extended specimens. Indeed, a specimen of *H. quissetensis* which was fixed in a moribund condition, is much extended and measures 14.2 mm. in length. The tissues had absorbed water and all the organs are larger than corresponding ones in well-fixed speci-

mens. The testes were almost smooth. The differences between *H. quissetensis* and *H. muehlensi* in size and in dimensions of particular organs are not surprising in view of the difference in hosts and condition of material. Indeed, Vogel noted that species of *Himasthla* had previously been reported only from birds and he raised the question whether *H. muehlensi* may not normally be a bird parasite which occasionally is introduced into the human intestine where it may become mature but in which it is probably retained for only a short time. Consideration of this problem and of the possible difference in number of spines is facilitated by certain observations of Beaver (1937). He found in *Echinostoma revolutum* that large individuals may produce accessory collar spines. He stated that ordinarily the worms do not attain a definitive size, but continue to grow until they are expelled. From the study of specimens developed in six different mammalian and three different avian hosts he reported, p. 28, "As a rule the worms develop more rapidly in birds (Table 7, p. 77), become mature earlier and at a smaller size, and live a much shorter period." The data of Beaver show that differences induced in individuals of the same species when reared in avian and mammalian hosts are as great as those between *H. quissetensis* and *H. muehlensi*.

The first experimental demonstration of the life cycle in the genus *Himasthla* supports the opinion of Vogel that the human species, *H. muehlensi*, is acquired by eating raw or insufficiently cooked mollusks. Examination of large numbers of *Venus mercenaria*, purchased in the New York market, has failed, however, to disclose the metacercaria of this species. Whether or not it is distinct from *H. quissetensis* remains to be determined.

Results of the present study on *H. quissetensis* supplement those of Beaver on *Echinostoma revolutum*. All of the gulls were fed approximately the same number of metacercariæ and 100 to 200 worms were recovered from the intestine of each of the birds killed two, three, twelve and thirty-one days after experimental feeding. The worms collected after thirty-one days in the intestine may not have been fully mature, since the terminal half of the uterus contained very few eggs. The bird sacrificed forty-five days after ingestion of cysts had been passing large numbers of eggs in the feces but contained only a single specimen when killed. It appears that in the present species the worms do not persist in the intestine very long after they become sexually mature.

Summary

Cercaria quissetensis Miller and Northup, 1926 has been restudied. It is the only echinostome cercaria which has been found in the

examination of over 18,000 specimens of *Nassa obsoleta* at Woods Hole. This cercaria encysts in the gills, mantle and foot of various mollusks. Metacercariæ were obtained experimentally in *Mya arenaria*, *Modiolus modiolus*, *Mytilus edulis*, *Cumingia tellinoides*, *Pecten irradians*, *Ensis directus*, and *Crepidula fornicata*. The metacercariæ are infective for birds three days after encystment and develop to maturity in the herring gull, *Larus argentatus*. Sexually mature specimens are identical with certain of those described by Linton (1928) as *Himasthla elongata* (Mehlis). Since there are important differences between the present specimens and *H. elongata*, they cannot be referred to that species and the name of the cercaria is adopted for them. Possible synonymy in the genus *Himasthla* is discussed.

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A METHOD FOR MAINTAINING A CONTINUOUS SUPPLY OF MARINE DIATOMS BY CULTURE[1]

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Culture techniques by means of which large supplies of unicellular organisms can be continually available are greatly in demand. This is especially true of unicellular plants, since they are convenient organisms for the study of photosynthetic and other metabolic processes.

The problem is essentially one of the maintenance of a growing population. So long as no factors develop which limit the rate of multiplication, increase in a culture or population is directly proportional to the number of organisms present. The growth of the population is logarithmic during the initial period. Some factor or factors in the environment, however, sooner or later lower the division rate. These factors may be limiting nutrient concentrations, formation of inhibitory excretory products, production of non-viable cells in the process of division, or, in the case of photosynthetic plants, limiting light intensity.

It was pointed out by Hjort, Jahn and Ottestad (1933), in a study of whaling in the antarctic, that the most advantageous way to exploit a population is to keep it at the level at which the greatest number of new organisms are produced in unit time. This paper presents an application of this principle to a culture method. After procuring the cell concentration at which the greatest daily yield is obtained the culture is maintained at this concentration. A volume of culture containing the quantity of cells equivalent to the daily increase is withdrawn each day and is replaced by an equal volume of fresh sea water enriched with nutrients.

We have used the marine diatom, *Nitzschia closterium*, which is an ideal organism for a study of culture technique. It is a small cell which is readily maintained in pure bacteria-free cultures, and which remains freely suspended in the sea water medium.

The culture medium used was that devised by Miquel for freshwater diatoms, and modified by Allen and Nelson (1910) for marine forms. We further modified it by the addition of $MgSO_4$ to the enrichment solutions.

[1] Contribution No. 172 from the Woods Hole Oceanographic Institution.

Sea water was enriched by the addition of nutrients prepared as follows:

Solution A	KNO_3	20.2 grams/100 cc. water
Solution B	$Na_2HPO_4(12\ H_2O)$	4 grams
	$CaCl_2$	4 "
	$MgSO_4$	4 "
	$FeCl_3$ (melted)	2 cc.
	Conc. HCl	2 cc.
	dilute to	100 cc. with water

To each liter of sea water 0.55 cc. of solution A and 0.5 cc. of solution B were added. The reduction of the quantity of solution B from 1 cc. as used by Allen and Nelson to 0.5 cc. per liter was made in order to eliminate the precipitation of iron, magnesium and calcium phosphate which forms when larger quantities are used. The amount of solution A was adjusted to give the usual ratio of phosphate phosphorus to nitrate nitrogen in sea water. This ratio, as reported by Redfield (1934), is about twenty atoms of nitrogen to one of phosphorus.

In the culture of unicellular plants it is difficult to obtain sufficient light intensity without undesirable heating. Neon tubes obviate this difficulty since they radiate very little heat, and the emission is close to the optimum for chlorophyll synthesis. The apparatus finally devised consists of a U-shaped neon tube which is surrounded by a glass water-jacket 8 cm. in diameter. This is placed in the axis of a large cylindrical museum jar 21.3 cm. in diameter by 90 cm. in height. The culture occupies the space between the two cylinders. The neon tube is completely surrounded by the culture and little light is lost except that which passes through the medium without being absorbed. By regulation of the flow of water through the water jacket the temperature in the culture can be controlled. Air containing 5 per cent CO_2 is bubbled through the culture to maintain a large source of available carbon, and to keep the cells from settling. Nutrient solutions may be added to the culture from time to time by means of a dropping funnel.

In flask cultures of *Nitzschia closterium* in the above medium the growth is limited to 2×10^9 cells per liter by the complete exhaustion of nutrients. In the large culture nutrients are added when necessary and ten times the maximum cell concentration produced in flask cultures can be obtained. Every time a number of cells sufficient to exhaust the nutrients is formed the appropriate amounts of the nutrient solutions must be added. The necessity for enrichment may

be determined by following the cell count or may be checked by periodical chemical analyses of the medium.

In Table I the data for the growth of a typical culture are pre-

TABLE I

Growth of a culture of twenty-six liters of Nitzschia closterium with regular enrichment of the medium.

Time	Cell count	Division rate (k) per day	Daily yield
days	*cells/liter*		*cells/liter*
0	0.004×10^9	—	—
2	0.043 "	1.19	0.02×10^9
3	0.140 "	1.15	0.10 "
5	0.52 "	0.655	0.19 "
6	1.04 "	0.530	0.52 "
7	2.10 "	0.530	1.06 "
9	3.58 "	0.27	0.75 "
11	8.10 "	0.40	2.25 "
13	11.5 "	0.18	1.7 "
14	12.8 "	0.11	1.3 "
15	13.8 "	0.07	1.0 "

sented. The division rate per cell is expressed by the velocity coefficient of the rate of growth, k. It is obtained from the equation

$$k = \frac{1}{t_2 - t_1} \cdot \ln \frac{C_{t_2}}{C_{t_1}}$$

in which C_{t_2} and C_{t_1} are respectively the cell concentrations at the end and beginning of the period under consideration. The division rate per cell remained approximately constant for the first three days of the culture period, after which it declined steadily.

The daily yield depends upon both the division rate and the cell concentration. It increased to a maximum on the eleventh day and then decreased. It is apparent that in order to obtain the maximum production of the culture it should be maintained at the cell concentration giving the optimum daily yield.

In Table II are given data procured by periodically reducing the cell concentration to the optimum by removal of the daily yield and subsequent dilution to the original volume. The values for the daily yield given in column five show that the production can be steadily maintained at its maximum. At the optimum cell concentration of 8×10^9 cells per liter 28 per cent of the total culture can be removed daily, giving a daily yield of about 2.4×10^9 cells per liter.

The total yield of a culture depends, of course, on its volume. The apparatus used in obtaining these data contained 26 liters, giving a total yield of about 62×10^9 cells per day. The dry weight of this quantity is approximately one gram. For the fifteen-day growth period of the culture (Table I) the average daily yield for the total period is only 24×10^9 cells per day. This value is only about one-third of that obtained by the renewal method after optimal conditions have been established and no continuous supply is obtained.

TABLE II

Yield of a culture of Nitzschia closterium maintained continuously at the optimal concentration for production.

Initial count	Final count	Duration of growth period	Division rate (k) per day	Daily yield
cells/liter	*cells/liter*	*hours*		*cells/liter*
8.84×10^9	11.36×10^9	25	0.243	2.42×10^9
7.70 "	11.15 "	41	0.217	2.02 "
8.10 "	12.92 "	45	0.250	2.57 "

This culture method was originally undertaken in an attempt to obtain diatoms for chemical analyses in quantities ranging up to a kilogram or more of dry material. The time required for producing such a quantity by this method could be reduced by increasing the number of units. A direct enlargement of the culture chamber is not desirable, since an increase in thickness of the culture would make the light intensity a serious limiting factor. A larger container could be used if it contained several light sources. A method for obtaining large quantities of diatoms directly from the sea has been described by Clarke (1937). Allen (1938) has recently criticized Clarke's method as being unnecessarily elaborate, but she does not appear to appreciate the magnitude of the quantities required, nor our need for a method applicable under those conditions of abundance frequently encountered in the sea.

The culture method described above has the advantage, not obtainable with natural collections, of producing a continuous supply of cells in a pure state. The culture can, moreover, be grown under various controlled environmental conditions and an ample quantity of material is produced daily for a variety of chemical and physiological tests. It is believed that a similar method can be applied advantageously to many other unicellular organisms required for physiological research.

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PARTHENOGENETIC MEROGONY OR DEVELOPMENT WITHOUT NUCLEI OF THE EGGS OF SEA URCHINS FROM NAPLES

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In a previous paper (1936) it was shown that non-nucleate parts of the eggs of *Arbacia punctulata*, obtained by centrifugal force, can be artificially activated and by successive cleavages they reach the blastula stage. This I have termed parthenogenetic merogony since it is a combination of parthenogenesis, in which the male nucleus is lacking, and merogony, in which the female nucleus is lacking; in parthenogenetic merogony both male and female nuclei are lacking, and the egg develops without any nuclei. In view of the fact that the non-nucleate halves of *Arbacia punctulata*, obtained by centrifugal force, are densely packed with pigment granules which apparently interfere somewhat with the formation of typical cell boundaries, even after fertilization, it seemed possible that further development of parthenogenetic merogones might be obtained with other species of sea urchin. Furthermore, the presence of mitochondria in the non-nucleate halves of certain species presented a means of testing the possibility that these granules might be helpful in development without nuclei. An investigation was carried out at Naples in the spring of 1937 and the results of the study are presented in this paper. Some observations on parthenogenetic and merogonic development are included, to serve as a basis for comparison of eggs without any nucleus (parthenogenetic merogones) with similar eggs and egg fractions having one nucleus, female or male (= "haplones"). A study has previously been made (1933) of the development of the centrifuged whole eggs and their nucleate fractions after fertilization, that is with two nuclei, both female and male (= "diplones"). Some observations on the effect of activating agents on immature eggs are also included since these bear on the general subject.

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Material and Method

The four species of sea urchins commonly occurring at Naples are *Arbacia pustulosa*, *Sphaerechinus granularis*, *Paracentrotus* (*Strongylocentrotus*) *lividus* and *Parechinus* (*Echinus*) *microtuberculatus*. The eggs of all these species can be broken apart by centrifugal force and these parts can be fertilized and will develop (1933). The nucleus always goes to the light pole, so that the heavy half of the egg is always non-nucleate. With further centrifuging, the halves can be broken into quarters. The non-nucleate fractions of the eggs of all four species can be artificially activated. The egg of *Parechinus microtuberculatus* proved the best one for the investigation since it breaks apart easily (thus avoiding heating while on the centrifuge) and the very clear protoplasm renders all phenomena easily visible in the living egg.

The most satisfactory parthenogenetic agent was the same as was used for *Arbacia punctulata*, i.e., sea water concentrated to about half its volume, or NaCl added to the sea water to bring it to a similar hypertonicity. After immersion for 10–20 minutes, the eggs were returned to sea water. Other methods were tried, especially Loeb's double method (butyric acid and a hypertonic solution) with less favorable results. One difficulty in the investigation has been to obtain a standard concentration and a definite period of immersion in the hypertonic solution owing to the extreme variability in reaction of different batches of eggs of the same species. Such has been the experience also of other investigators in obtaining parthenogenesis of entire eggs.

Stratification by Centrifugal Force

When the eggs of any of the four species are centrifuged, the nucleus always goes to the light pole under the oil cap. This is invariable in mature unfertilized eggs. The stratification of materials, however, is different in the different species, thus causing a difference in granular content of the non-nucleate fractions of the different species; these fractions also differ in size in comparison with their corresponding nucleate fractions. The stratification of the four species is shown in Photographs 1–13.

The normal egg of *Arbacia pustulosa* is exactly like that of *Arbacia punctulata* except that it is a little larger (79 μ as vs. 74 μ) and more heavily pigmented; and it stratifies similarly. The stratification of *Arbacia pustulosa* (Photograph 1) is:—oil at the light pole, then a clear layer, then a band of mitochondria (small granules which stain purple with methyl green), then yolk granules, and then red pigment granules at the heavy pole. In the other three species pigment granules are

lacking, except for the faint (occasionally quite reddish) band encircling the *Paracentrotus* egg, which seems to be a definite structure not moved by the centrifugal forces used in my experiments. This is probably due to the fact that the pigment granules forming the band are embedded in the cortical protoplasm which is generally conceded to have a higher viscosity than the interior. The egg of *Arbacia pustulosa* breaks apart in 3 minutes at 10,000 × g into two almost equal halves, the non-nucleate half containing only yolk and pigment and no mitochondria (Photograph 2). With further centrifuging, the halves can be broken into quarters (Photograph 3).

The normal egg of *Sphaerechinus granularis* averages 98 μ in diameter. The stratification under centrifugal force is similar to that of *Arbacia* except for lack of pigment granules; i.e., oil, clear layer, mitochondria and yolk (Photograph 4). There is frequently a clear layer also at the centrifugal pole below the yolk (Photograph 5). The egg breaks apart at 10,000 × g in 5 minutes (Photograph 6). The non-nucleate fraction is much larger than the lighter nucleate fraction and contains yellowish yolk granules and a few mitochondria. With further centrifuging both half-eggs can be broken into quarters.

The eggs of the other two species differ from those of *Arbacia* and *Sphaerechinus* in having the mitochondria heavier than the yolk granules. The egg of *Paracentrotus lividus* has an average diameter of 90 μ. The usual stratification under centrifugal force is:—oil, clear

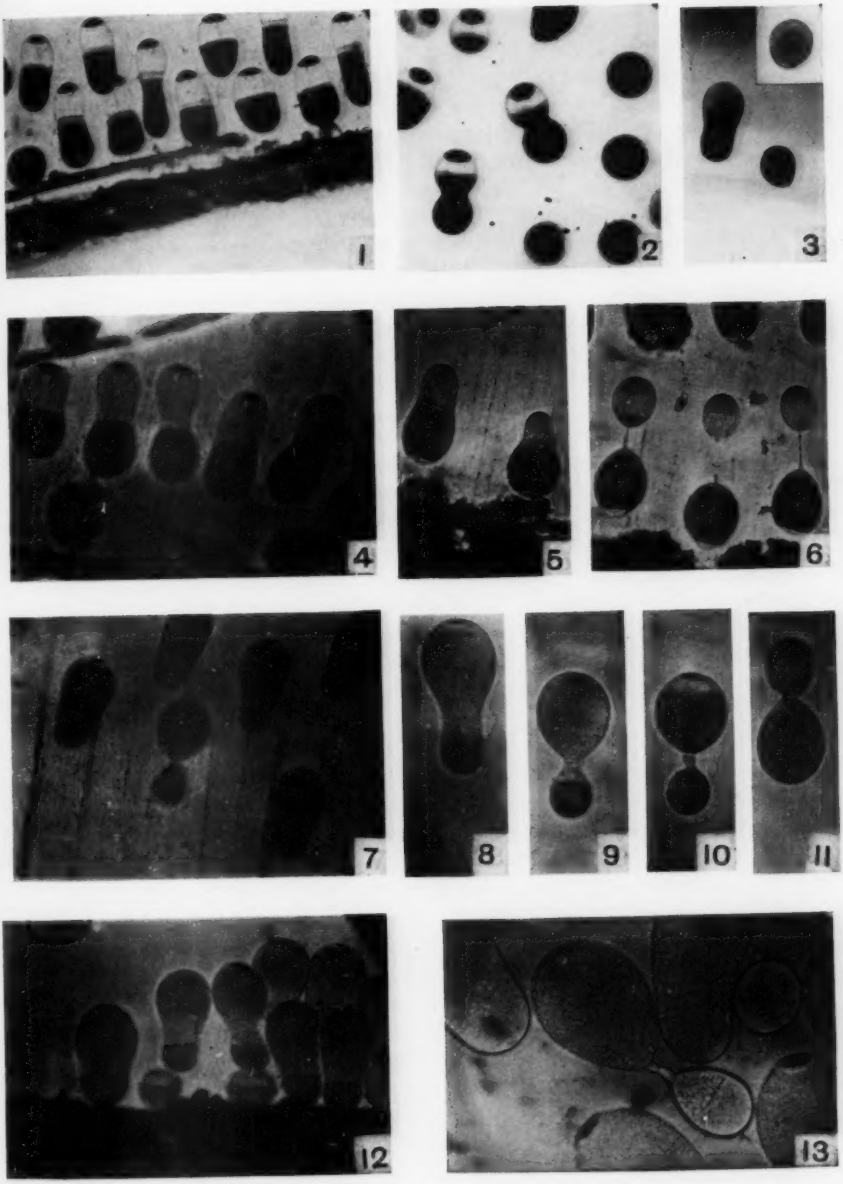
PHOTOGRAPHS 1–13

Stratification and Breaking of Eggs

The photographs were all taken of the living eggs with a Leica camera. Photographs 1, 4, 5, 6, 7, 12 were taken of eggs in a centrifuge microscope slide immediately after being rotated on the centrifuge microscope, and all with the same magnification, so that the sizes of the eggs as pictured are comparative.

PHOTOGRAPH 1. *Arbacia pustulosa*—Stratification.
PHOTOGRAPH 2. *Arbacia pustulosa*—Stratified whole and half-eggs.
PHOTOGRAPH 3. *Arbacia pustulosa*—Red half and its two quarters.
PHOTOGRAPH 4. *Sphaerechinus granularis*—Stratification.
PHOTOGRAPH 5. *Sphaerechinus granularis*—Stratification showing clear layer at bottom of eggs.
PHOTOGRAPH 6. *Sphaerechinus granularis*—Breaking apart.
PHOTOGRAPH 7. *Paracentrotus lividus*—Usual stratification.
PHOTOGRAPH 8. *Paracentrotus lividus*—Stratification with clear layer at bottom of egg.
PHOTOGRAPH 9. *Paracentrotus lividus*—No clear layer at top of egg (Like *Parechinus*).
PHOTOGRAPH 10. *Paracentrotus lividus*—Breaking into large upper and small lower halves.
PHOTOGRAPH 11. *Paracentrotus lividus*—Breaking into small upper and large lower halves.
PHOTOGRAPH 12. *Parechinus microtuberculatus*—Stratification.
PHOTOGRAPH 13. *Parechinus microtuberculatus*—Breaking apart.

PLATE I



layer, yolk, clear layer and mitochondria (Photograph 7). There is, however, some variation in the position of the clear layer; in some eggs there is no clear layer between the yolk and mitochondria; in some eggs there is a clear zone below the mitochondria (Photograph 8). And sometimes there is no clear layer below the oil (Photograph 9); in this case the stratification is like that of *Parechinus* (Cf. Photographs 9 and 12). The clear substratum is apparently, therefore, not uniform, but must be of graded density throughout its extent in the centrifuged egg. It takes 30 minutes at 10,000 × g to break these eggs apart; they are much more difficult to break apart than the eggs of the other species. There are two types of breaking. The egg may break into a larger nucleate fraction and a small non-nucleate sphere, as the majority of eggs did in the springs of 1933, 1934 and 1937 (Photograph 10) or they may break into a smaller nucleate fraction and a large non-nucleate fraction as the majority of eggs did in 1932 (Photograph 11) (E. B. Harvey, 1933). The two types must be due to something inherent in the egg as they may occur in eggs from the same female centrifuged together in the same tube, although any set of eggs tends to be either of the one type or the other. A similar peculiarity has been noted previously for *Arbacia punctulata* in unusual batches of eggs (1936). In either case, the non-nucleate sphere of *Paracentrotus* contains all the mitochondria and some of the yolk granules.

The egg of *Parechinus microtuberculatus* is the largest of the four species; its average diameter is 102 μ. The typical stratification is:—oil, yolk granules, clear layer, mitochondria (Photograph 12). The nucleus, therefore, lies typically among granules at the centripetal pole and not in a clear layer as in the eggs of the other species of sea urchins and of most other eggs. There is occasionally, however, also a clear layer under the oil in which case this egg resembles the typical *Paracentrotus* egg. The egg breaks apart in 8 minutes at 10,000 × g, and with further centrifuging the lower half can be broken into quarters. The non-nucleate halves are much smaller than the nucleate, and contain mitochondria and practically no other granules (Photograph 13).

We see, then, that the eggs of the four species of sea urchin under consideration stratify differently, and that therefore the non-nucleate fractions contain different kinds of granules. In the non-nucleate halves of *Arbacia*, pigment granules are present, in the other three species, they are not. In *Sphaerechinus*, *Paracentrotus* and *Parechinus*, mitochondrial granules are present, in *Arbacia*, they are not. In *Parechinus* there are practically no yolk granules, but these are present in the non-nucleate halves of the other three species. The clear protoplasm which is the matrix in which granules are suspended is, of

course, present in all the non-nucleate halves, in greater or less amount, and appears as a definite layer when free of granules, as is typical for *Parechinus*.

Arbacia Pustulosa

Parthenogenetic Merogones

The non-nucleate red halves, yolk quarters and pigment quarters of *Arbacia pustulosa* have been activated artificially (= parthenogenetic merogones). They throw off a fertilization membrane closely adhering to the surface and form a thick ectoplasmic layer, as is characteristic of the normal fertilized egg of this species. A large monaster appears in many of the fractions about two hours after activation, succeeded in another hour or so by an amphiaster; these are usually visible only in the yolk quarters and the less pigmented part of the red halves. After another hour, a cleavage plane may come in between the two asters. This has been observed to come in chiefly in the long axis of the elongate red half-eggs and to extend only to the pigmented area. Some of the red halves have reached by successive cleavages a 16-cell stage with good cell boundaries. Photographs 14–17 are successive photographs of the same two eggs. In many red halves, cell boundaries do not come in, but the egg may be literally peppered with small asters (Photograph 18). In many of the red half-eggs, a cleavage takes place at the pigmented end (Photographs 19, 20). In the yolk quarters which are usually spherical, the first cleavage plane divides the fraction equatorially or nearly so (Photograph 21). No further development has been obtained of the parthenogenetic merogones of *Arbacia pustulosa*, but it seems likely that with a more extensive study, blastulæ could be obtained similar to those of *Arbacia punctulata*.

Haplones

The term "*haplone*" is used to include eggs which develop with only one nucleus whether female (= parthenogenetic) or male (= merogonic, i.e. a fertilized egg fragment from which the female nucleus has been removed) and therefore containing a haploid set of chromosomes. The normal fertilized egg, containing both male and female nuclei and therefore a diploid set of chromosomes, is termed a "*diplone*."

The normal egg of *Arbacia pustulosa* may be activated by parthenogenetic agents, and also the whole egg after it has been centrifuged, as well as the white half which contains the nucleus. They all throw off a closely adherent fertilization membrane characteristic of the normal fertilized egg. The cleavage is similar to that of the fertilized egg except that it takes place at a slower rate. The first cleavage of the artificially activated egg, both normal and centrifuged, takes place only

after 3 hours (at 20° C.), often much longer, whereas that of the fertilized egg takes place in 1⅓ hours. The two-cell stage of the elongate centrifuged egg, both fertilized and parthenogenetic, is similar to that of *Arbacia punctulata;* the first cleavage plane usually comes in across the short axis of the egg, in the less dense granular area, dividing the egg into a smaller cell with few granules and a larger pigmented cell (Photograph 22). The first cleavage plane of the spherical white half, both fertilized and parthenogenetic, is equatorial (Photograph 23). Parthenogenetic whole eggs, both normal and centrifuged, and parthenogenetic white halves have given swimming blastulæ, but were carried no further.

The non-nucleate halves and quarters when fertilized (= fertilized merogones) have sometimes cleaved quite regularly, when spherical, but at a slow rate (Photograph 24); but often, as in *Arbacia punctulata*, cell boundaries fail to come in, giving rise to multi-nucleate eggs. Nuclei are often plainly visible in these fertilized merogones (Photograph 25). They contrast strikingly in this respect with the parthenogenetic merogones in which asters may be present but not nuclei. A few swimmers have been obtained but many of the fertilized merogones fail to develop.

Parechinus Microtuberculatus

Parthenogenetic Merogones

The non-nucleate halves of *Parechinus microtuberculatus* consist of mitochondrial granules and clear layer (Photograph 26). When they

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Photographs 14–25

Arbacia pustulosa

Photographs 14–21. Parthenogenetic merogones. Photographs 22–25. Haplones.

Photograph 14. Two red half-eggs, 4½ hours after activation. Two asters were plainly visible in the egg at left.

Photograph 15. Same, 5 minutes later. Cleavage between the two asters.

Photograph 16. Same, 1 hour later (5½ hours after activation). Note cleavage plane appearing in egg at right.

Photograph 17. Same, 1/2 hour later (6 hours after activation).

Photograph 18. Red half-egg, peppered with asters (7½ hours after activation).

Photograph 19. Red half-egg, 5¼ hours after activation.

Photograph 20. Similar red half-egg, 22 hours after activation.

Photograph 21. Yolk quarter, 4½ hours after activation. The cleavage plane was observed to come in between two large asters.

Photograph 22. Whole egg centrifuged, then activated, 6 hours after activation.

Photograph 23. White half-egg, 6 hours after activation.

Photograph 24. Fertilized red half, 5¼ hours after fertilization.

Photograph 25. Fertilized yolk quarter, 9½ hours after fertilization. Observe nuclei in the cells.

PLATE II

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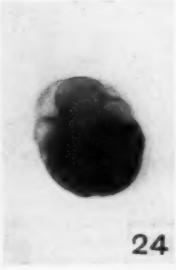
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are activated, they throw off fertilization membranes widely separated from the surface of the egg, leaving a large perivitelline space just as is characteristic of the normal fertilized egg of this species (Photograph 27). The membranes are frequently thrown off while in the hypertonic solution and the surface of the egg then becomes irregular or crenate (Photograph 28). On return to sea water, these eggs swell and the perivitelline space is often completely obliterated; the fertilization membrane is frequently broken by further swelling. A large monaster forms three or four hours after activation and after another hour or so there may be two, three or more asters. Cleavage planes have been observed coming in around the asters, resulting in two-, three-, or four-cell stages (Photographs 29, 30, 31). No regular succession of cleavages has been observed, such as occur in *Arbacia*. The egg seems somewhat labile and cleavage planes appear and disappear. These eggs later break up into a number of small cells, becoming progressively smaller and more numerous, often while still within the fertilization membrane, resembling a blastula. Photographs 31, 32 and 33 are successive stages of the same egg showing the breaking up, following normal cleavage planes (Photograph 31) which had been observed to come in between three large asters. Photographs 34, 35 and 36 are successive stages of another egg. Many of these blastulæ occur as well as other blastulæ in which there are no cell boundaries; these may be filled with asters. The blastulæ emerge from the fertilization membranes in quite typical fashion on the day after activation, leaving the ruptured empty membranes (Photograph 37) and they may live for several days. The characteristic breaking up of the non-nucleate half-egg might be looked upon as stages in degeneration rather than as stages in development. But an exactly similar process takes place also

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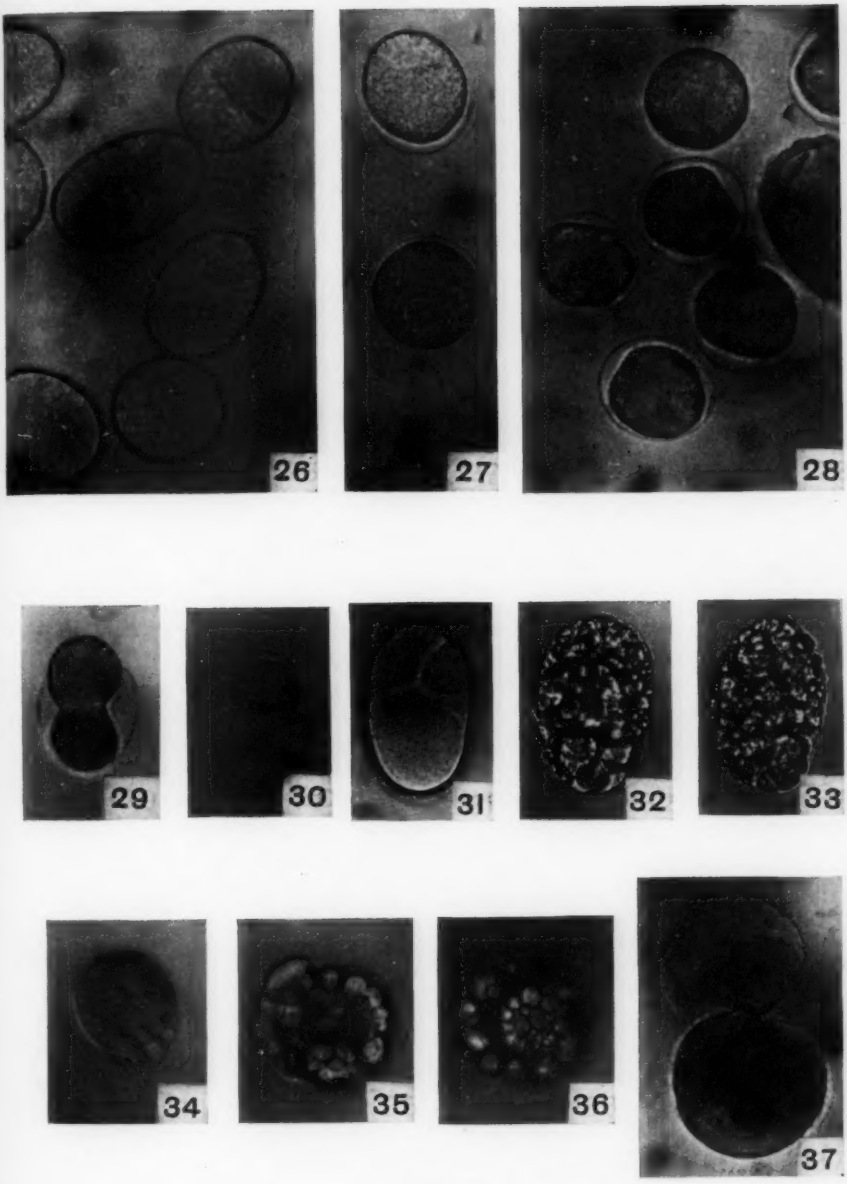
PHOTOGRAPHS 26–37

Parechinus microtuberculatus. Parthenogenetic merogones.

PHOTOGRAPH 26. Non-nucleate lower halves, just broken off by centrifuging.
PHOTOGRAPH 27. Ten minutes after activation, to show fertilization membranes.
PHOTOGRAPH 28. Non-nucleate halves still in hypertonic sea water for 1/2 hour.
PHOTOGRAPH 29. Beginning two-cell stage, still in hypertonic sea water for three hours.
PHOTOGRAPH 30. Two-cell stage, seven hours after activation.
PHOTOGRAPH 31. Three-cell stage, four hours after activation; three large asters were plainly visible but do not show in the photograph.
PHOTOGRAPH 32. Same egg as 31, five hours later.
PHOTOGRAPH 33. Same egg, one hour later (ten hours after activation).
PHOTOGRAPH 34. Another egg, twenty-two hours after activation.
PHOTOGRAPH 35. Same egg, five hours later.
PHOTOGRAPH 36. Same egg, six hours later (thirty-three hours after activation).
PHOTOGRAPH 37. Non-nucleate egg emerging from fertilization membrane, twenty hours after activation.

PLATE III



in the whole egg leading to normal blastulæ as described below. The similarity may be readily seen by comparing Photographs 34, 35 and 36 with Photographs 39, 40 and 42.

Haplones

Normal uncentrifuged eggs of *Parechinus microtuberculatus* were treated with hypertonic sea water for twenty-five minutes, then transferred to sea water. Fertilization membranes formed and after some hours the nuclei enlarged as they normally do previous to breaking down. In Photograph 38, taken seven and a half hours after activation, the enlarged nuclei may be seen in the two eggs to the left, and in the right-hand egg, the nuclear membrane has just broken (it was observed to break) and the cell has become somewhat amoeboid. Fifteen minutes later, this egg has begun to break up into a number of irregular cells (Photograph 39) and the process has continued further in Photograph 40, taken fifteen minutes later. The process continues as is shown in Photographs 41 and 42, until a blastula is formed, often quite normal in appearance, consisting of many cells of the same size. After some hours the blastulæ break through the fertilization membranes and become free-swimming, with cilia. Some of these seem quite regular and normal but some are abnormal consisting of cells of varying sizes. Many normal gastrulæ later developed, presumably (not actually observed) from the blastulæ which had developed in this irregular fashion (Photograph 43). A comparison of Photographs 34 with 39, 35 with 40, and 36 with 42 will show the similarity of the breaking up of the non-nucleate egg with the process in the nucleate parthenogenetic egg which gives rise to some quite normal-looking swimmers.

Some centrifuged whole eggs treated with the parthenogenetic agent cleaved similarly to such eggs after fertilization. In one set of

PHOTOGRAPHS 38–46

Parechinus microtuberculatus. Haplones.

PHOTOGRAPH 38. Normal eggs, 7½ hours after activation. Nuclear membrane had just broken in egg to right.

PHOTOGRAPH 39. Same eggs, 25 minutes later; egg breaking up irregularly.

PHOTOGRAPH 40. Same eggs, 15 minutes later.

PHOTOGRAPH 41. Similar eggs, 7½ hours after activation.

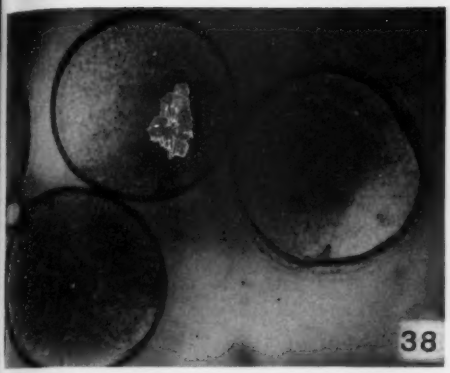
PHOTOGRAPH 42. Similar eggs, 7½ hours after activation. Note perfect blastula.

PHOTOGRAPH 43. Similar eggs, 24 hours after activation. Note perfect gastrula.

PHOTOGRAPH 44. Centrifuged egg; cell division, 15 minutes after activation, while nucleus is still intact.

PHOTOGRAPH 45. Similar eggs, 5 hours later; upper part cytolyzed.

PHOTOGRAPH 46. Fertilized merogone, 2 hours after fertilization.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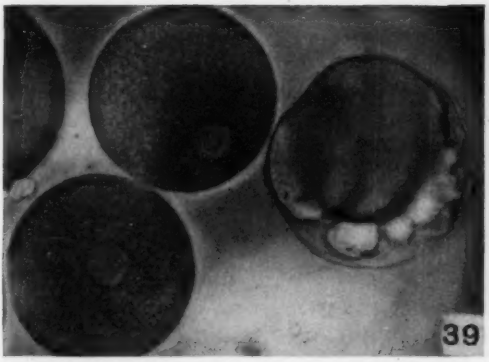
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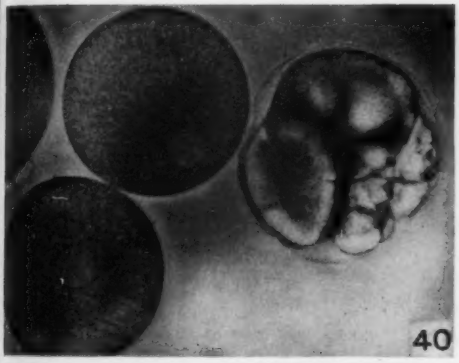
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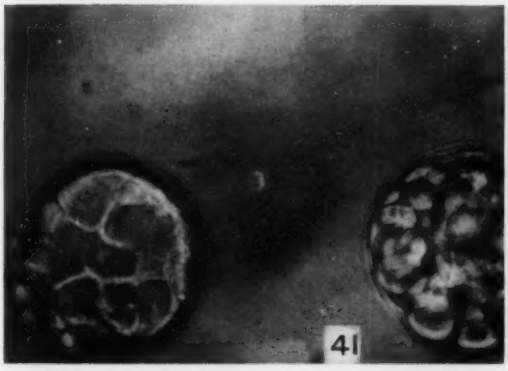
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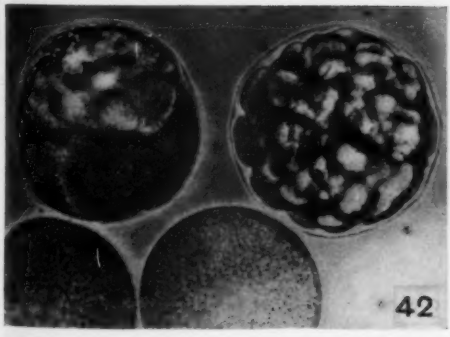
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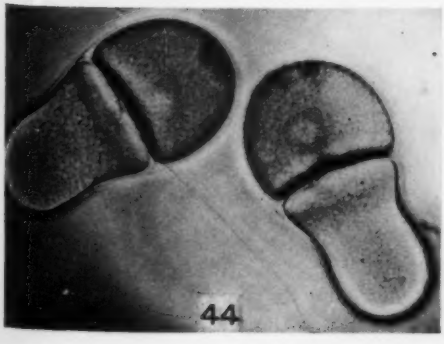
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parthenogenetic centrifuged eggs, however, a cleavage plane came in fifteen minutes after activation while the nucleus was still intact (Photograph 44). This came across the short axis of the egg, producing the same two-cell stage characteristic of the elongate *Arbacia* egg, both fertilized and parthenogenetic (Cf. Photograph 44 with 22, and with 30, 31 and 36 of my 1936 paper). This is another instance of cleavage planes coming in independently of any nuclear changes and emphasizes the fact that the cleavage of an egg and the nuclear changes usually accompanying it are quite separate phenomena. Later on, the upper part of these eggs cytolyzed, leaving the lower part with mitochondrial granules, intact (Photograph 45).

The fertilized merogones in many cases cleaved quite regularly. Photograph 46 is an eight-cell stage showing nuclei.

Paracentrotus lividus

Parthenogenetic Merogones

The development of the non-nucleate fractions of *Paracentrotus* after activation is exactly like that of *Parechinus* described above. The fertilization membrane is thrown off, leaving a large perivitelline space characteristic of the normal fertilized egg of this species. A conspicuous large aster is formed (Photograph 47) followed by two or more smaller asters. A few early cleavages have been observed (Photograph 48) but the cleavage planes come and go and then the egg breaks up into a number of cells becoming progressively smaller and more numerous, just as in *Parechinus*, giving rise to a blastula-like embryo (Photographs 49, 50).

Photographs 47–58

Photographs 47–51. *Paracentrotus lividus.* Photographs 52–58. *Sphaerechinus granularis.*

Photograph 47. *Paracentrotus lividus.* Non-nucleate half, 1½ hours after activation. Note large monaster.

Photograph 48. Beginning 2-cell stage, 4 hours after activation.

Photograph 49. Same egg as 47, 2½ hours after activation; egg fragmenting.

Photograph 50. Same egg 1/2 hour later.

Photograph 51. Parthenogenetic centrifuged whole egg, tripartite. Note aster in the stalk and many asters in upper part.

Photograph 52. *Sphaerechinus granularis.* Non-nucleate half, 2 hours after activation.

Photograph 53. Same egg, 3 hours later.

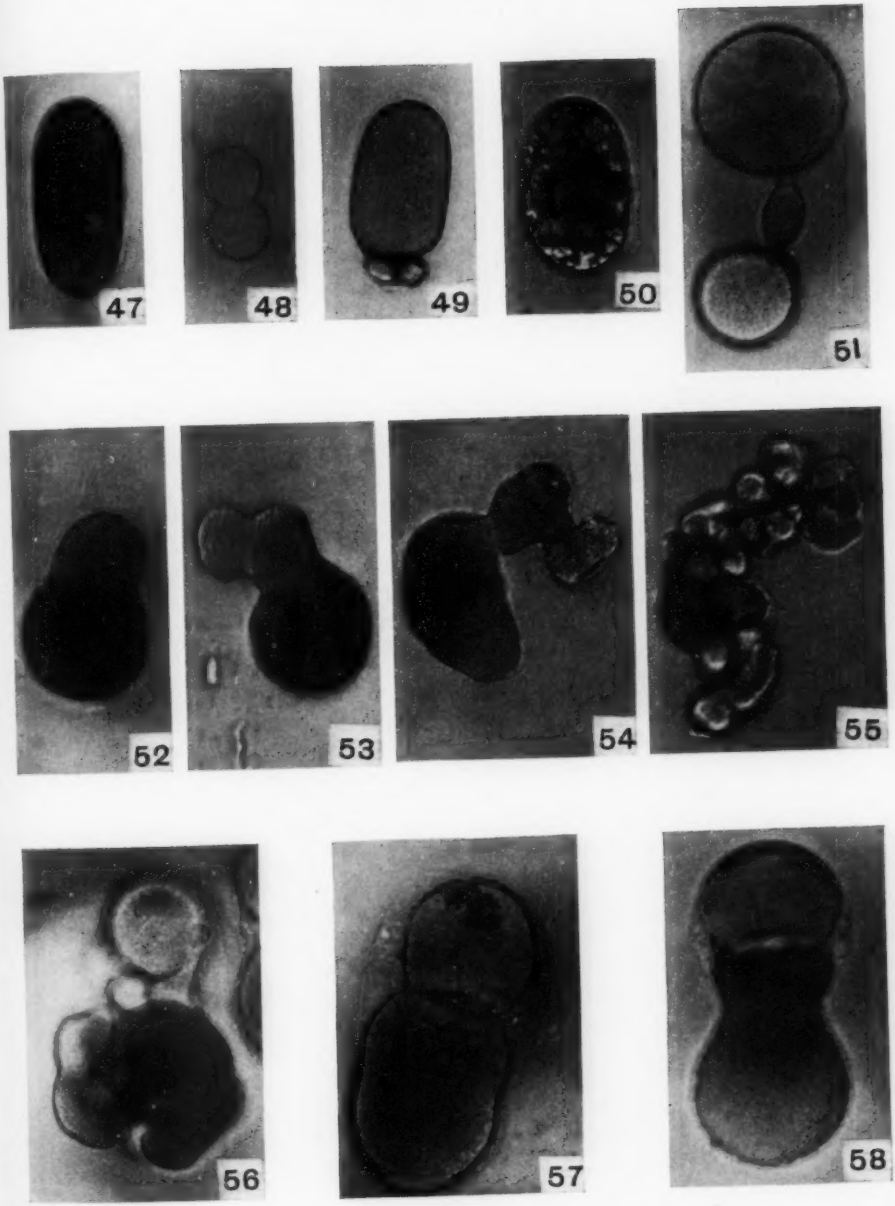
Photograph 54. Same egg, 1/2 hour later.

Photograph 55. Same egg, 1 hour later.

Photograph 56. Parthenogenetic centrifuged whole egg, 5 hours after activation.

Photographs 57, 58. Cleavage of parthenogenetic centrifuged whole eggs while nucleus is still intact; 1 hour after activation.

PLATE V



Haplones

In the normal egg and in the centrifuged whole egg treated with hypertonic solutions, asters frequently appear in the protoplasm while the nucleus is still intact. Attention has previously been called (1933) to the peculiar tripartite eggs which develop after fertilization of a centrifuged egg, giving rise to three embryos. The middle portion of these eggs is simply the thickened stalk between the two half-eggs when incompletely broken apart. In a similar parthenogenetic egg, the fertilization membrane forms also around the stalk and a monaster appears in the stalk, even before the nucleus in the upper half has broken down. This is shown in Photograph 51 in which the upper part is filled with many asters (light areas in the photograph). Later on, the upper nucleate part of the centrifuged egg becomes cytolyzed, while the lower part may remain intact and even cleave. The cleavage of the lower part may also take place while the nucleus is intact in the upper part.

Swimmers have been obtained from parthenogenetic normal eggs but not from centrifuged whole eggs or the white nucleate halves after parthenogenetic reagents.

Good cleavages of the fertilized merogones have been obtained. Owing to the long period of centrifuging (thirty minutes) necessary for breaking apart the *Paracentrotus* egg, and the consequent heating up of the machine and the eggs, investigation of this species has been limited.

SPHAERECHINUS GRANULARIS

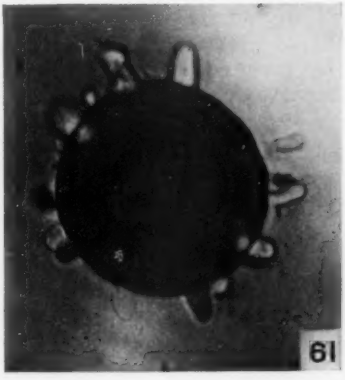
Parthenogenetic Merogones

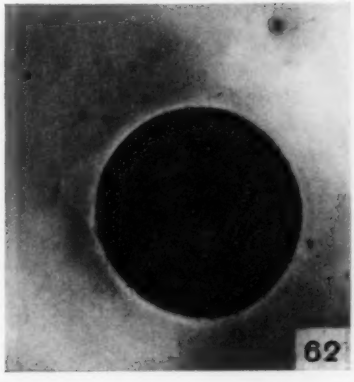
The non-nucleate half of the *Sphaerechinus* egg, although larger in proportion than that of the other eggs studied and containing both mitochondria and yolk granules, does not develop as well. Fertilization membranes well separated from the surface, as is characteristic of the normal egg, are thrown off, often while still in the hypertonic solution. The egg then becomes amœboid, and fragments irregularly into a number of pieces. The irregularity of this process may be seen from Photographs 52–55 which are consecutive pictures of the same egg.

Haplones

The normal egg and the centrifuged whole egg after being subjected to parthenogenetic agents throw off fertilization membranes but do not go far in development. The centrifuged whole egg usually becomes

PLATE VI





PHOTOGRAPHS 59–62

Immature eggs of *Parechinus microtuberculatus*.

PHOTOGRAPH 59. Centrifuged egg, almost broken apart. Note thin layer of material over both parts and over connecting strand.

PHOTOGRAPH 60. Change of shape and breaking up of normal immature egg, one hour after hypertonic sea water.

PHOTOGRAPH 61. Normal immature egg in sea water showing protrusions wherever sperm have hit the surface.

PHOTOGRAPH 62. Immature egg after hypertonic sea water and return to sea water with sperm; no protrusions on the surface.

amœboid and breaks into a number of fragments just as the non-nucleate fraction does (Photograph 56).

A peculiar phenomenon was observed in the centrifuged whole eggs and the white (nucleate) halves kept for a long time (one hour) in the hypertonic solution. The nucleus became elliptical, the long axis of the ellipse always parallel with the planes of stratification. As it later moved down through the clear layer it moved with its long axis in any direction. The ellipse almost immediately rounded out into a sphere when transferred to sea water. The cause and meaning of the ellipse is not known. An elliptical nucleus has been observed also in the centrifuged eggs of *Arbacia punctulata* after fertilization, but here it seems to occur at a definite stage in development, just prior to its breaking down (See Fig. 28 of my 1932 paper).

A cleavage plane in the parthenogenetic centrifuged egg frequently comes in across the short axis of the egg (but not usually at its narrowest part) while the nucleus is still intact (Photographs 57, 58) just as in *Parechinus*, showing again that a cleavage plane may be independent of nuclear changes.

The fertilized merogones cleave and develop quite normally, as previously described (1933).

Immature Eggs

Since the immature egg differs from the mature in having a large amount of material in the germinal vesicle, which mixes with the egg substance on maturation, it would be of great interest to get the non-nucleate portion of an immature egg to develop. This has been tried, and although not successful, some interesting phenomena have been observed.

(1) Immature eggs incompletely broken apart by centrifugal force were put in a hypertonic solution. While still in the solution, a thin layer formed on the outside of both the nucleate and non-nucleate parts and also on the connecting strand (Photograph 59). No fertilization membrane was formed, but the layer of material resembled the ectoplasmic layer characteristic of mature eggs activated either by fertilization or by parthenogenetic agents.

(2) When normal uncentrifuged immature eggs were treated with a hypertonic solution, they became elongate, with the germinal vesicle at one end; the other end pinched off a small piece. Subsequently this lower piece divided into two and later into many parts (Photograph 60). The same result was obtained with centrifuged immature eggs. Though this is not a typical cleavage, the process is similar and indicates some sort of change in the surface similar to that occurring during a true cleavage of a mature egg.

(3) The fact that the surface of immature eggs is definitely changed by the parthenogenetic agent is shown by their reaction to sperm. The normal immature egg forms characteristic protrusions at the surface wherever the sperm hit (Photograph 61); these resemble enlarged fertilization cones. Immature eggs which have been in hypertonic sea water and are then put back into normal sea water and sperm added, do not form these protrusions or react in any way to the sperm (Photograph 62).

Discussion

Although a further investigation of parthenogenetic merogony in four other species of sea urchin has not resulted in obtaining further development after the blastula stage, it has shown that the possibility of activating non-nucleate parts of eggs is fairly general. It may be that eggs will go only as far as the blastula stage without nuclei, and that nuclear material is necessary for differentiation, perhaps furnishing both chemical substances and genes.

All the non-nucleate parts will throw off a membrane, which is characteristic of the normal fertilized egg of its species, and they will all form an ectoplasmic layer and asters. The cleavage and early development does not seem to be dependent on any particular type of granule, for any type,—yolk, pigment or mitochondria, may be lacking in the different species and the egg still cleave. Cleavage is not dependent on a nucleus, for it may take place when it is completely absent or when it is present but intact and apparently inert. Nor is it dependent upon the presence of a spindle and asters as these may be centrifuged to another part of the cell and the cleavage plane come in without relation to them, as shown in a previous paper (1935). Cleavage seems to be dependent rather on the physical and chemical state of the protoplasm itself and of its surface layer. An orderly succession of cleavages is not necessary for the production of a many-celled organism. The cytoplasm can break up irregularly into fragments both when nuclear material is present and when it is not, and yet give rise to a blastula quite similar to that obtained by successive cleavages. The lability of cleavage planes and their disappearance and reappearance is characteristic of some of these non-nucleate fractions, just as was found for normal eggs deprived of oxygen (E. B. Harvey, 1927). Cleavage, budding and fragmentation seem to be different expressions of the same state of cytoplasm or of the cell surface, or both, in its effort to subdivide itself into smaller entities. Whatever the cell with a nucleus has been observed to do in this respect, the same cell without a nucleus has been observed to do, at least for a certain period. At

the time of cleavage, a tendency for the egg to become amœboid seems to be an expression of the physical state of the surface, just as the appearance of asters is usually associated with the state of the interior protoplasm at this time.

Summary

1. The stratification, by centrifugal force, of the eggs of the four species of sea urchin occurring at Naples is described.

2. The non-nucleate parts of the eggs of all four species can be activated by parthenogenetic agents. They throw off fertilization membranes characteristic of the species, and begin to develop.

3. Development of the parthenogenetic merogones of *Arbacia pustulosa* is similar to that of *Arbacia punctulata*.

4. The parthenogenetic merogones of *Parechinus* and *Paracentrotus* develop by a process of progressive fragmentation inside the fertilization membrane, similar to that occurring in the parthenogenetic normal egg which leads to normal free-swimming blastulæ.

5. Cleavage may take place in whole eggs while the nucleus is still intact.

6. Certain changes take place in the immature egg similar to those of the mature egg when treated with parthenogenetic agents.

7. No further development of eggs without nuclei has yet been obtained beyond the blastula.

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AN EXPERIMENTAL STUDY ON MITOSIS IN THE SOMATIC CELLS OF WHEAT[1]

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In the normal mitotic division of a cell the fundamental processes are seen as constituting a perfectly integrated whole. However, occasionally in nature and frequently under experimental or diseased conditions, these basic coördinated processes which occur in both the nucleus and cytoplasm become dissociated or suppressed, resulting in a failure to divide or abnormal division of the cell.

There are numerous agencies which have been reported as modifying or inhibiting cell division, namely: cold, heat, anæsthetics, basic dye-stuffs, injury, disease, poisons, hypertonic and hypotonic solutions, centrifuging, various types of radiation and hybridization. Recently, geneticists have made use of these methods in an effort to induce polyploidy in both normal and hybrid species of plants with the hope of producing more profitable economic varieties.

Our interest in this problem rests primarily in its cytological aspects, that is, to show how colchicine in certain concentrations and centrifuging at high speeds may affect the normal mitotic mechanism in rapidly dividing cells and, in addition, to analyze the mechanism that may eventually give rise to polyploids under experimental conditions.

Material and Methods

The variety of wheat which we have used in all experiments is known as "Red Durham." A large number of seeds were first soaked from 3 to 5 hours in tap water and from these only the most uniform in size and appearance were selected and placed in a moist chamber where they were allowed to germinate at 20° to 22° C. in the dark. After approximately 16 to 20 hours the growing parts of the wheat embryo were observed bursting through the surface of the swollen seeds. Some of the germinating seeds were then placed in various concentrations of colchicine ranging from 1 : 250 to 1 : 2000 for 30 minutes to 12 hours. After treatment with colchicine they were washed in tap water and fixed immediately or allowed to stand in a moist chamber for various intervals before fixing.

Others of the germinating seeds were placed in the rotor of the air turbine ultracentrifuge (Beams, Weed, and Pickels, 1933) and centri-

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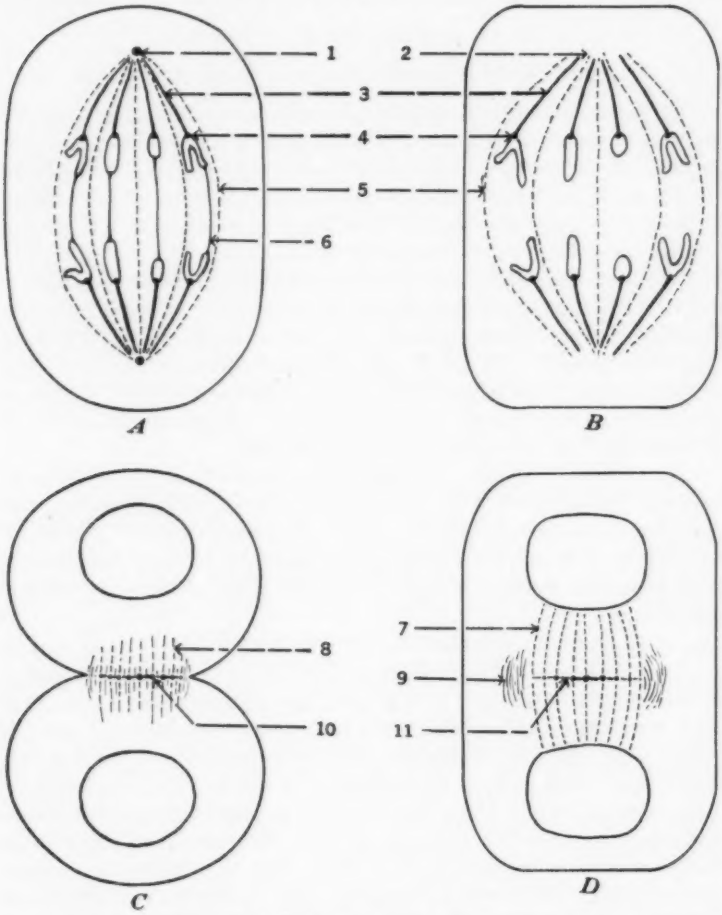
fuged at various speeds giving forces from 15,000 to approximately 100,000 times gravity. The best procedure for our studies has been to centrifuge the seeds at 100,000 times gravity for 10 minutes, after



TEXT FIG. 1. Diagrams showing the mitotic components in certain plant and animal cells. *A*, *C*, animal cells; *B*, *D*, plant cells. 1, centriole; 2, polar center; 3, traction, half-spindle, centromere, or chromosome fiber; 4, fiber attachment, centromere or kinetochore; 5, continuous spindle fiber; 6, interzonal fiber; 7, spindle of cytokinesis; 8, spindle remnant; 9, phragmoplast; 10, mid-bodies; 11, cell plate.

which they were either fixed immediately or after they had recovered for varying lengths of time.

All material whether colchicine-treated or centrifuged has been fixed in Bouin's solution and subsequently stained in Heidenhain's hæmatoxylin.

Description

General

That a mitotic spindle can be differentiated from the surrounding cytoplasm during cell division in many animal and plant cells seems amply demonstrated. However, many questions concerning the origin, structure and function of the different elements of the spindle remain unanswered. Early studies upon the mitotic spindle and current elementary textbooks of biology generally describe it as composed of a single set of fibers. However, in animal cells it has been demonstrated to consist of a number of elements definitely correlated to function as a single unit (Text Fig. 1, *A–C*). In plants, too, it can be demonstrated that the spindle is made up of a number of different components derived from the nucleus, from the cytoplasm or from both (Text Fig. 1, *B–D*). No centrosomes or asters can be demonstrated in the higher plants comparable to those in many animal cells. However, physiological poles or centers where the spindle fibers converge seem to perform much the same function as do the centrosomes in animal cells. As far as we have been able to determine, no interzonal fibers comparable to those found in animal cells have been clearly shown for plants. Moreover, in the cells of higher plants the continuous fibers are usually described as giving rise to the "spindle of cytokinesis" and the phragmoplast which eventually forms the cell plate; in certain animal cells the continuous fibers appear to give rise to mid-bodies (see Fry, 1937, for a recent discussion on this point).

The Effects of Colchicine

The effects of colchicine upon dividing cells have been studied by a number of investigators (Dustin, 1934; Lits, 1934, 1936; Ludford, 1936; Brues and Cohen, 1936; Allen, Smith, and Gardner, 1937; Blakeslee and Avery, 1937; Havas, 1937; Brues and Jackson, 1937; Okkels, 1937; Richards, 1937; Nebel and Ruttle, 1938).[2] It seems to change the colloidal nature of the cytoplasm, thus inhibiting the physico-chemical changes that normally accompany cell division. In this way the achromatic spindle elements are prevented from forming, and the chromosomes are left stranded at the metaphase (Fig. 2). If, however, the cells are sufficiently affected by the colchicine, the divided chromosomes will break down and form a restitution nucleus with double the somatic number of chromosomes present. In other words, the nuclear changes characteristic of mitosis proceed more or

[2] See also, Walker, R. I., 1938. *Am. Jour Bot.*, **25**: 280.

less normally to metaphase, where chromosome movements are arrested much longer than usual. After an abnormally long delay at metaphase the chromosomes which are double separate, but only for a comparatively short distance because of the absence of spindle fibers, and an ameboid nucleus is finally formed (Figs. 3, 33). Under suitable exposure to colchicine the same cell may pass through several cycles of this type, giving rise to a doubling of the chromosomes at each cycle (Figs. 6, 7, 29, 30). At each division of the chromosomes the cell body enlarges, as well as the nucleus, until the roots and shoots of the embryos so treated have grown greatly in diameter, simply as a result of the enlargement of the cells without division. The cytoplasm of such cells is greatly vacuolated and the nucleus, in addition to containing an abundance of chromatin and, frequently, vacuoles also, possesses numerous nucleoli. We have counted as high as 75 nucleoli in the nucleus of a cell of this type. In fact, the appearances of such cells in many ways are strikingly similar to those of certain tumor cells (compare with the figures of Levine, 1931). Cells affected to this extent seldom recover.

Tissues that are exposed to suitable concentrations of colchicine for the proper time readily recover. For instance, those that have

EXPLANATION OF FIGURES

All drawings were made by Miss Bernette Bohen from colchicine-treated cells of the roots of germinated wheat seeds.

FIG. 2. Cell arrested at metaphase. The chromosomes which have divided but which have not separated are much more loosely arranged than those in control cells at the same stage.

FIG. 3. Restitution nucleus resulting from a condition as in Fig. 1. An incomplete cell wall is shown.

FIG. 4. Binucleate cell.

FIG. 5. Binucleate cell undergoing mitosis.

FIG. 6. A "resting" cell that has passed through two or more divisions of the nucleus only.

FIG. 7. Metaphase of a cell as in Fig. 6. Many more than the normal number of chromosomes are present.

FIG. 8. Late anaphase showing a tripolar spindle.

FIG. 9. Metaphase plate showing numerous constrictions of the chromosomes. Not all the chromosomes in the cell are shown.

FIG. 10. One form of abnormal separation of the chromosomes in a cell that had partially recovered from colchicine treatment.

FIG. 11. Three cells derived from a single tripolar spindle as in Fig. 8.

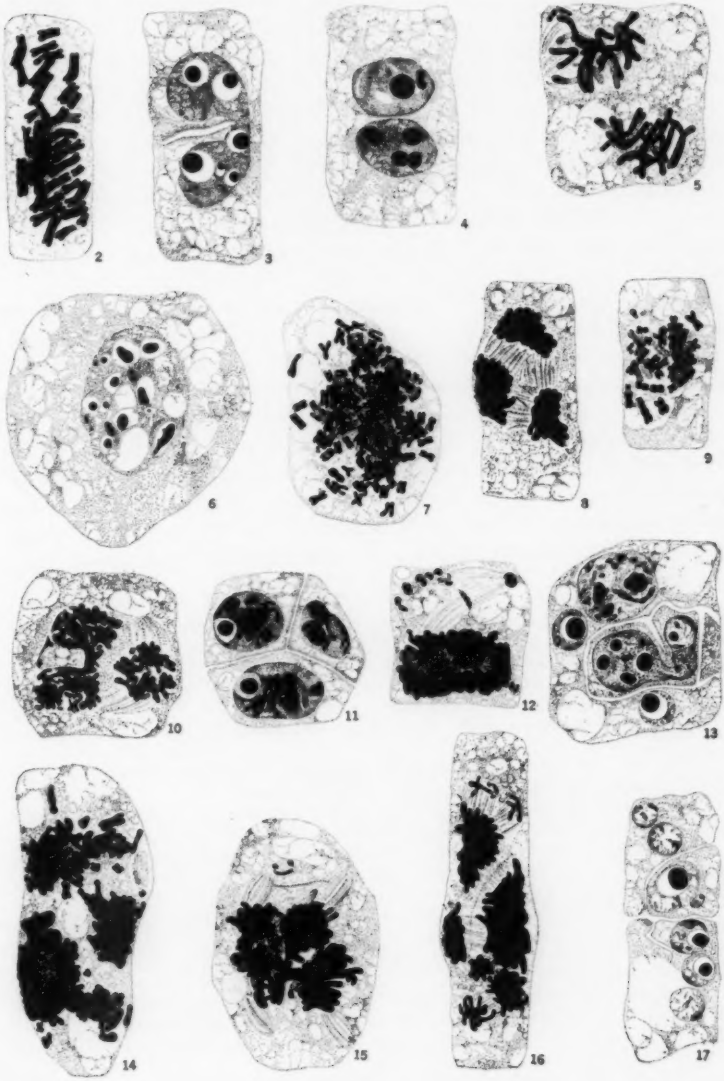
FIG. 12. Cell showing small spindles between the scattered individual chromosomes in a partially recovered cell.

FIG. 13. Three irregular cells. The large U-shaped peripheral one contains one large nucleus and two smaller ones. The central one contains an abnormally large bean-shaped nucleus, the other cell being a small non-nucleated one.

FIGS. 14, 15, and 16. Dividing cells with more than the normal number of somatic chromosomes. Various types of abnormalities that occur in the separation of the chromosomes are shown.

FIG. 17. One example of an abnormal cell that has resulted from an abnormal mitotic division similar to those illustrated in Figs. 14, 15, and 16.

PLATE I



passed through one mitotic cycle of the nucleus only may completely recover and, at the next mitosis, form a normal achromatic spindle mechanism and divide with double the somatic number of chromosomes. It is the production of this and similar conditions that the geneticists are striving to attain. If, on the other hand, the effect of the colchicine treatment has been such that the cell recovers after a long arrest of the chromosomes at metaphase, many types of mitotic abnormalities may arise. Because of the great number of chromosomes in our material it has not been possible to accurately count them. Nevertheless, with reasonable certainty it is possible to determine those cells that have formed some multiple of the normal diploid number.

In different regions of the same root in which mitosis has been arrested by colchicine we have found the chromosomes to vary from apparently the normal condition to that in which the chromosomes possess numerous constrictions (Fig. 9). Cells blocked at metaphase sometimes have their chromosomes abnormally scattered on the "equatorial plate" (Fig. 2). In fact, as pointed out by Brues and Cohen (1936), the chromosomes appear as though they actually repelled each other. Perhaps this condition is due to the absence of the controlling forces of the spindle fibers. When recovery takes place and the spindle or spindles start to appear, the loosely arranged chro-

EXPLANATION OF FIGURES

All figures were drawn by Miss Bernette Bohen from centrifuged cells of the roots of germinated wheat seeds. The centrifugal force in all cases has been directed approximately toward the bottom of the plate.

FIG. 18. Prophase, showing chromatin displaced to the centrifugal end of the nucleus.

FIG. 19. Metaphase, with chromosomes and spindle displaced to centrifugal end of the cell.

FIG. 20. An unusual case where the chromosomes have been thrown free of the metaphase spindle. Not all the chromosomes are shown.

FIG. 21. Late anaphase with most of the chromosomes separated from the spindle. The chromosomes are displaced centrifugally, the spindle centripetally.

FIGS. 22, 23, and 24. Cells in telophase, showing displacement of nuclei. No spindle fibers or phragmoplast were observed in these cells. They had evidently disintegrated.

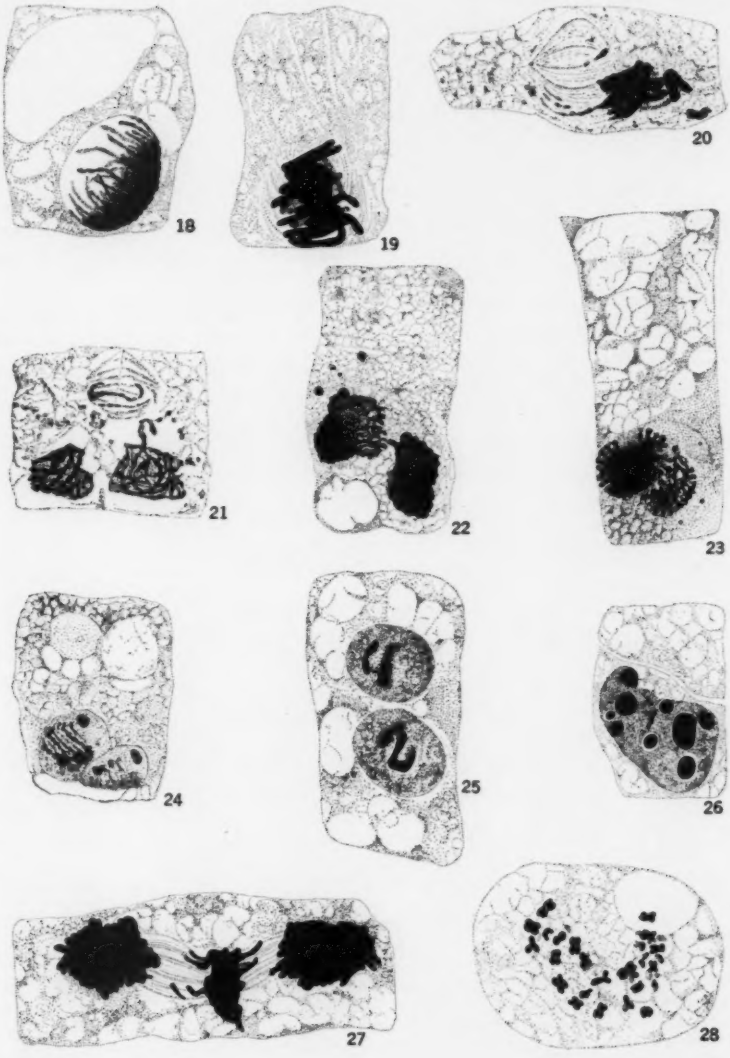
FIG. 25. Binucleate cell resulting from a condition like that in Figs. 21, 22, 23, and 24.

FIG. 26. A condition in our material where the chromosomes have been thrown centrifugally, and upon recovery have formed a restitution nucleus. Cytokinesis has taken place giving rise to one daughter nucleated cell (with a double set of somatic chromosomes present) and one daughter non-nucleated cell.

FIG. 27. Tripolar cell. Cells of this kind give rise to three cells with an unequal number of chromosomes.

FIG. 28. Cell showing constricted chromosomes that are shortened and thickened. This condition probably represents an injury effect. Not all the chromosomes in the cell are shown.

PLATE II



mosomes have a tendency to segregate into groups, each of which apparently induces, or, at least, is associated with a definite spindle. The chromosomes in each group are often quite unequal in number (Figs. 12, 14, 15, 16, 32, 35, 37), thus giving rise to nuclei and cells of unequal size (Figs. 11, 13, 17, 31, 32, 34). If recovery has been incomplete and the spindles poorly developed, the chromosomes do not separate normally and no cell plate, or an incomplete one, is formed between the nuclei, depending apparently on the degree of recovery.

As previously mentioned, when no spindle is present the daughter chromosomes separate only a comparatively short distance and a restitution nucleus which is frequently greatly elongated or lobed is formed. If the chromosomes separate a greater distance during anaphase and telophase, generally a binucleate cell results, the nuclei often being of unequal size depending upon the equal or unequal partition of the chromosomes (Fig. 4). These cells may later form two metaphase plates (Figs. 5, 36). In cells like Figs. 3 and 33 an incomplete cell wall is formed. This condition has apparently resulted from a failure of the cell wall to cut through the large bean-shaped restitution nucleus which was formed before the cell wall was laid down. Many

Explanation of Figures

Photomicrographs of colchicine-treated and centrifuged cells from young roots of germinated wheat seeds. Figures 29 to 39 inclusive, colchicine-treated; Figs. 40 to 47 inclusive, centrifuged.

Fig. 29. Cell that had passed through two or more mitotic cycles of the nucleus only. Large ameboid nucleus containing many nucleoli is shown.

Fig. 30. Division of a cell like that shown in Fig. 7. Many more than the normal number of chromosomes is present.

Fig. 31. Five cells formed from an irregular mitosis as shown in Fig. 17.

Fig. 32. Three cells in mitosis which have resulted from division of a single cell.

Fig. 33. Same cell as drawn in Fig. 3.

Fig. 34. Four cells of unequal size derived from a single abnormal mitosis as shown in Fig. 31.

Fig. 35. Cell dividing abnormally with three groups of chromosomes; same cell as Fig. 14.

Fig. 36. Two sets of metaphase chromosomes of unequal size in same cell.

Figs. 37, 38, and 39. Other forms of multipolar conditions.

Figs. 40 and 41. Centrifuged cells showing displacement of chromosomes in prophase (40) and metaphase (41).

Fig. 42. Late anaphase showing displacement of mitotic figure. Bits of fragmented chromatin are seen in the centripetal end of the cell.

Fig. 43. Telophase showing the displaced nuclei at the centrifugal end of the cell; the phragmoplast has not been displaced. Such a cell appears to give rise to a condition like that shown in Fig. 26.

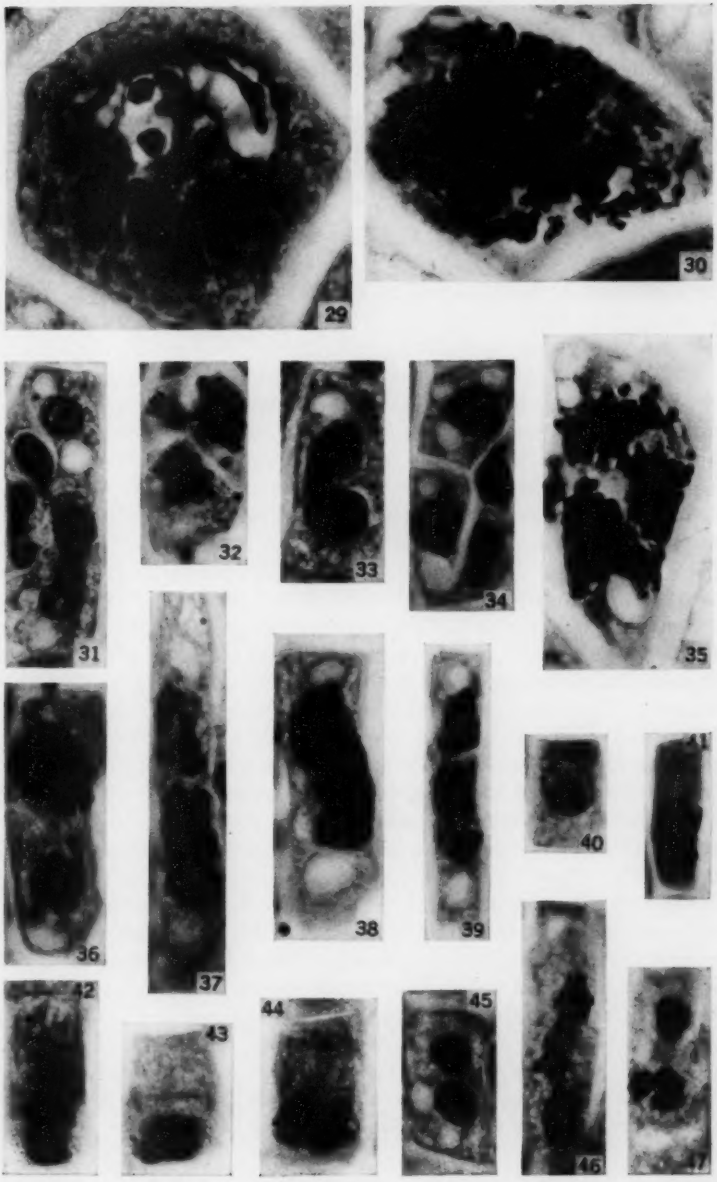
Fig. 44. Cell centrifuged at a slightly earlier stage in the mitotic cycle than that in Fig. 43. Here the phragmoplast is seen displaced to the centripetal end of the cell.

Fig. 45. Binucleate cell.

Fig. 46. Same cell as shown in Fig. 27.

Fig. 47. Another type of mitotic abnormality similar to that shown in Fig. 46.

PLATE III



such instances of this and similar conditions were observed in our material.

Abnormal mitoses like those illustrated in Figs. 8, 10, 12, 14, 15, 16, 32, 35, 37, 38, and 39 obviously give rise to cells with various chromosome numbers. It is highly probable that cells of this type included in a plant with normal or polyploid cells that have recovered from the colchicine treatment divide for only a short time and eventually disappear. However, we have not made a definite study of this point.

Effects of Ultracentrifuging

Plant cells and tissues have been centrifuged by several workers (Mottier, 1899; Andrews, 1915; Němec, 1915, 1929; Schaede, 1930; Kostoff, 1935, 1937, 1938; Luyet, 1935; Luyet and Ernst, 1934; Beams and King, 1935; etc.).

The general effect of strong centrifugal force (150,000–400,000 times gravity) upon the nucleus and cytoplasm of plant cells, i.e., the stratification of the materials in the order of their relative specific gravity, has been recently reported by us and need not be discussed here (Beams and King, 1935).

During prophase the chromosomes are displaced by ultracentrifuging and sometimes fragmented in the nucleus as illustrated in Figs. 18 and 40. In the metaphase many different effects of the ultracentrifuging are noted; for instance, in Figs. 19 and 41 the whole metaphase plate of chromosomes has been displaced to the centrifugal pole. The spindle is usually greatly distorted and in some cases seems to have disappeared completely. Just how the cell recovers from this condition is not altogether clear since we are unable to observe them in the living condition. However, in many instances at least, they apparently form a more or less normal anaphase and telophase except that the division takes place in the centrifugal end of the cell (Fig. 42). If this condition persists, it results in great discrepancy in size of the resulting daughter cells. In addition to displaced mitotic figures, many cells are seen with bits of fragmented chromatin resting free in the cytoplasm (Figs. 22, 42). In other cells which were fixed in anaphase following recovery from centrifugation in the metaphase multipolar spindles are present (Figs. 27, 46, 47). These spindles were probably formed as a result of the dispersal of the chromosomes by centrifuging. Conditions of this kind give rise to cells of unequal size and chromosome number. The chromosomes in relatively rare cells are shortened, thickened, and show various constrictions (Fig. 28), probably an indication that they have been injured as noted above. We have observed similar constrictions following colchicine treatment.

In a few cells which had been exposed to 100,000 times the force of gravity the chromosomes have been thrown free of the spindle (Fig. 20). Sometimes bits of chromatin which presumably contain the kinetochore or centromere remain attached to the spindle. This condition, which is relatively rare in wheat cells, is not uncommon in centrifuged embryonic chick cells (Beams and King, 1936). In anaphase and telophase stages also the chromosomes may be completely separated from the spindle (Fig. 21). In this cell the chromosomes have been thrown centrifugally while the spindle with a few bits of chromatin has been displaced centripetally. Here, then, as in other cells (Schrader, 1934; Luyet, 1935; Beams and King, 1935; and others), the chromosomes are heavier and the spindle substance is lighter than the surrounding cytoplasm.

In like manner, cells centrifuged in later telophase usually have their chromosomes or developing nuclei displaced to the centrifugal end, the "spindle of cytokinesis" and phragmoplast to the centripetal end of the cell (Figs. 22, 23, 24, 44). This condition almost invariably gives rise to a binucleate cell (Figs. 25, 45). Whether cytokinesis takes place in cells of this type apparently depends upon whether or not the phragmoplast had actually started to differentiate a cell plate. If the cell plate has started to appear in the center of the spindle, it is usually not greatly displaced (Fig. 43). Such a cell undoubtedly gives rise to a condition such as that shown in Fig. 26. Sometimes instead of the two displaced nuclei fusing as in Fig. 26, they remain distinct, thus forming one daughter cell with two nuclei, the other daughter cell being without a nucleus. If, however, the nuclei are displaced at a slightly earlier stage (Fig. 44), before the cell plate has definitely started to form, the "spindle of cytokinesis" is displaced to the centripetal end of the cell. In such cases no cell plate or wall is formed and a binucleate cell results much in the same way as that described by Němec (1929) in cells treated with nicotine. Both nuclei in cells of this kind form metaphase plates and divide simultaneously. If the spindles are situated close to each other, they apparently fuse in a manner suggested by Jørgensen (1928) but we have never actually observed this condition.

Discussion

There are numerous agents (see introduction) that will allow the cells to undergo karyokinesis without the usual accompanying cytokinesis, but none are more suitable for this purpose than the narcotic alkaloid colchicine. As pointed out by Ludford (1936), Dixon (1905) seems to have been the first to observe many cells in division after treatment with colchicine. He concluded that its effect was to "excite

karyokinesis." However, Dustin (1934) and Lits (1934) were the first to suggest that the effect of the drug was that of a mitotic poison. More recently papers by Ludford (1936), Brues and Cohen (1936), Allen, Smith, and Gardner (1937), Blakeslee and Avery (1937), Havas (1937), Brues and Jackson (1937), Nebel and Ruttle (1938) and others have shown that colchicine specifically inhibits the formation of an achromatic spindle so that mitosis is blocked at metaphase. After a long delay at metaphase the chromosomes do not separate normally and cytokinesis does not take place, thus giving rise to a double number of chromosomes within the cell. We have been able to substantiate these general facts in this paper. In addition, the recovery stages of the cell from the colchicine treatment are interesting when viewed in the light of the mechanism of mitosis.

The fact that an incompact metaphase plate is formed in colchicine-treated cells without spindles indicates that the spindle fibers exert a definite force which bears upon the arrangement of the chromosomes at this stage. Also, when the chromosomes are loosely arranged at metaphase and allowed to recover, separate spindles appear in connection with the various groups of chromosomes, regardless of their size. Such conditions would seem to indicate that in this material the chromosomes are capable of organizing or inducing a spindle. (However, see the recent work of Gross, 1936, and Harvey, 1936.) It is a fact of interest that in colchicine-treated cells the arrested metaphase chromosomes are apparently incapable of forming the traction or centromere fibers which certain observers have described for the normal condition (Darlington, 1936; Carothers, 1936).

Richards (1937) has shown that the effects of colchicine on yeast are to stimulate growth, instead of arresting it, as is the case in wheat and in many other tissues. Unpublished observations indicate that the division rate of *Paramecium multimicronucleatum* is not decreased by colchicine in concentrations up to $\frac{1}{4}$ per cent. These observations are in agreement with the general view that the action of colchicine on mitosis is to inhibit the development of the achromatic spindle, since it is generally believed that yeast divides by amitosis.

It has long been held (see Sharp, 1934, for literature) that the cell plate in higher plants is formed directly from the "spindle of cytokinesis" and phragmoplast which are thought to be a product of the continuous fibers of the spindle, although this is denied by Robyns (1926). If this condition is true, then it is easy to understand why no cell plate or cell wall is formed in colchicine-treated material. Incomplete cell walls that sometimes form are, no doubt, in some way due to the abnormal development and function of the phragmoplast in

partially recovered cells. When the chromatic (chromosomes) and achromatic (spindle and polar centers) elements are thrown out of step at metaphase by the late appearance of the achromatic elements the chromosomes usually proceed to form a restitution nucleus. The abnormally late appearance of the phragmoplast in relation to the chromosome cycle, while sometimes capable of giving rise to a cell plate and wall, is unable to cut through the restitution nucleus. It would seem, therefore, that the mechanism that produces cytokinesis is incapable of cutting through the nuclear substance.

It is now definitely known from the works of a number of authors (see Heilbrunn, 1928, for literature) that mitotic division is accompanied by definite changes in the viscosity of the cytoplasm, which are essential for spindle formation. Most of the methods that have been used to inhibit cell division or dissociate karyokinesis from cytokinesis seem to do so by preventing these normal viscosity changes. By centrifuging normal and colchicine-treated material at the same speed for the same time we have attempted to determine whether or not an effective dose of colchicine changes the viscosity of the cytoplasm. As a result we have found that the colchicine-treated cells show a more marked displacement of the chromosomes than does the control material, particularly at the metaphase stage. This indicates that a lowering of the viscosity in the colchicine-treated cells has taken place. However, it should be pointed out, as shown by Nebel and Ruttle (1938) for *Tradescantia* cells that cyclosis is not noticeably affected by the colchicine treatment. Colchicine in suitable concentrations to arrest mitosis in wheat cells does not prevent the normal cyclosis in *Paramecium*, nor the changes in viscosity associated with locomotion in Ameba.

In certain grain hybrids various observations suggestive of gonomery as described for certain animals (Wilson, 1925) have been published by Bleier (1930), who concludes that not only do species differ as to behavior of chromosomes, but also as to that of their spindle substance. This, he thinks is closely associated with the chromosomes—he even suggests that each chromosome may have individual spindle substance which is different from that of other chromosomes of the same species. Darlington and Thomas (1937), who have published on similar hybrids, believe that there are two spindles, one derived from the cytoplasm (spindle) and one from the fiber attachment of the chromosomes (centromere spindle). Normal behavior in mitosis involves the integration of these two, but either may appear independently. In hybrids the normal relationship of these is disrupted, with resulting abnormal divisions much as in Bleier's hybrids.

Various experimental treatments will cause similar abnormal divisions in somatic cells of non-hybrid material as shown by our observations.

Wasielewski (1903, 1904) reported that somatic cells treated with chloral hydrate divide amitotically. Němec (1904) repeated the experiments of Wasielewski and was able to confirm, in general, his observations but could not agree with his interpretation that chloral hydrate caused the cells to divide by amitosis. On the other hand, Němec showed that the treatment with chloral hydrate produced an arrested mitosis resulting in many types of mitotic abnormalities. Němec further suggested that the polyploid condition frequently resulting from treatment with chloral hydrate eventually disappeared from the tissue by a process of heterotypical reduction comparable to that occurring in spore mother cells. This latter assumption has not been generally confirmed. Kemp (1910) and Sakamura (1920) particularly have made extensive studies of the action of chloral hydrate upon dividing cells. In general they report that its effects on dividing cells is to induce mitotic abnormalities which are not unlike those described in this paper.

Van Regemorter (1926) attributes the failure of normal spindles to form in chloralized cells to a destruction of the cell's polarity; recovery from the effect of the reagent involving a return to the proper polarized condition of the spindle substance.

Numerous methods for the production of polyploidy in somatic cells have been suggested by plant breeders, of which the following may be listed: (For a review of the literature on this subject see Winkler, 1916; Jørgensen, 1928; Lindstrom and Koos, 1931; Bhatia, 1938.)

1. The failure or incomplete separation of the chromosomes at anaphase with the formation of a restitution nucleus with double the normal number of chromosomes present.

2. The formation of a binucleate cell, due to the failure of development of the cell wall between the two resulting nuclei or its later disintegration. Some authors describe the nuclei as subsequently fusing and others as remaining separate.

3. The fusion of two neighboring cells into one, usually the result of injury in grafting experiments or to disease.

4. The union of the two metaphase spindles of a binucleate cell to form one giant one ("endo-duplication").

It will be evident from our description that we have observed in colchicine-treated and in centrifuged material all the conditions mentioned in (1) and (2) as well as variations of them. We have never observed a fusion of neighboring cells after the cell plate had definitely formed. Neither have we observed a fusion of metaphase spindles of

binucleate cells, although we may assume that it does occur. We have observed what appeared to be a fusion of two separately induced spindles of the loosely arranged metaphase chromosomes in cells that had been treated with colchicine.

It should be pointed out that the normal development of the spindle in higher plants seems to differ somewhat from that in animal cells. Some investigators believe, as pointed out by Lawson (1900) and Allen (1903), that the spindle in higher plants instead of being bipolar from the first and under the control of definite polar centers passes through a series of multipolar stages in the course of its development. Accordingly, in higher plants the looseness or incompactness of the spindle fibers, particularly at the polar centers, as compared to the condition in animal cells, may possibly offer an explanation as to why in the apparently normal tissue of plants more polyploid cells and irregular mitoses have been found than has been reported for animal cells.

As previously pointed out, both plant and animal cells have been centrifuged by several workers (see Kostoff, 1938, for a recent review of the literature). In general it has been shown that the cell materials stratify in the order of their relative specific gravities. In most cases the chromosomes, particularly during mitosis, are displaced to the centrifugal end of the cell. Kostoff (1938) has been able to induce alterations in somatic chromosome sets of various plants. After centrifuging in an ordinary laboratory centrifuge he found that various results, such as the failure of the chromosomes to separate, of the cell to divide, and injury to the spindle, as well as the displacement of single chromosomes, have given rise to monosomic, trisomic, polysomic, and various other forms of polyploid cells.

For the most part, our work confirms that of Kostoff (1938) and others on the general results of centrifuging dividing cells. In addition, certain effects of centrifuging on the "spindle of cytokinesis" and phragmoplast in relation to cell plate formation should be considered here.

As seen above, there is good evidence that the "spindle of cytokinesis" and phragmoplast give rise to the cell plate. According to Timberlake (1900) and others the cell plate is formed by a fusion of swellings occurring on the middle portion of the continuous spindle fibers. More recently it has been emphasized that the cell plate appears as a continuous film among the spindle fibers but not on them (Sharp, 1934, for references). However, the origin of this material which collects at the equator, and forms the cell plate is unknown but it has been claimed, according to Sharp (1934), by Dembouiski and

Ziegenspeck (1929), Damehl and Ziegenspeck (1929) to be derived from the nucleus.

Notwithstanding the fact that in some plant material the cell plate forms independently of the spindle fibers, there is every reason to believe that in the typical condition the "spindle of cytokinesis" induces cell plate formation. This is especially clear from the work of Jungers (1931) on the endosperm of Iris. This is multinucleate, and after the last nuclear division, secondary spindles arise on the flanks of the spindles connecting daughter nuclei. These secondary spindles connect the nuclei with neighboring nuclei. The phragmoplasts grow out from the connecting spindles and meet in the secondary spindles, thus bringing about the inclusion of single nuclei within cell walls. In our material, if the spindle fibers and phragmoplast are displaced early enough in the cycle, no cell membrane or wall appears and the displaced spindle and phragmoplast eventually disintegrate. Accordingly, we conclude that the material forming the cell plate has been displaced in the centrifuge toward the centripetal end of the cell, where it rarely, if ever, forms a wall. This is interesting in view of our recent work on the eggs of *Ascaris* (Beams and King, 1937) where the cleavage substance ("surface active material") is displaced by centrifuging, thus preventing cytokinesis although karyokinesis takes place more or less normally.

Conclusions

1. Colchicine markedly interferes with the dynamics of cell division. Dividing cells are arrested for an abnormally long time at metaphase, at which time the chromosomes become loosely arranged and sometimes segregate into groups. No spindle or polar center is formed so that the daughter chromosomes do not separate normally. However, eventually they form a single large restitution nucleus or occasionally two nuclei. In either case, no cell plate or wall is formed if the treatment has been sufficient, thus giving rise to double the somatic number of chromosomes within the cell. Some cells may pass through several cycles of this kind.

2. Cells that have partially recovered from the colchicine treatment, particularly at metaphase, show many other types of mitotic abnormalities. We have counted as high as 7 spindles arising from one incompact set of metaphase chromosomes. Separate spindles may be induced by the abnormal grouping of the chromosomes at metaphase, regardless of the size, of the single groups. Such abnormal spindles occurring in a single cell eventually give rise to several daughter cells, usually of different sizes.

3. Evidence is presented which indicates that the effect of the colchicine treatment is to lower the viscosity of the cytoplasm, thus inhibiting the normal viscosity changes that accompany mitosis. This in turn prevents the formation of the polar centers and achromatic spindle.

4. The use of colchicine is an excellent method to disengage the nuclear from the cytoplasmic processes in mitosis; a condition which would seem to indicate that they are independent in causation but related in effect.

5. Centrifuging at high speeds stratifies the cell materials in the order of their relative specific gravity. Cells may recover from such treatment.

6. In a few cells at metaphase the chromosomes are thrown free of the achromatic spindle. Usually, however, the whole spindle is displaced to the centrifugal end of cell. Recovery and subsequent division in such cells vary from the normal condition to that in which multipolar spindles are present. Such figures give rise to more than the normal number of daughter cells.

7. Cells centrifuged while in anaphase or telophase before the cell plate has begun to form, have the chromosomes or developing nuclei displaced to the centrifugal end of the cell; the "spindle of cytokinesis" or developing phragmoplast to the centripetal end of the cell, where it seems finally to disintegrate. This condition usually gives rise to a binucleate cell.

8. Evidence is presented that the cell plate-forming substance or organizer is displaced in much the same manner as is the cleavage substance in the centrifuged eggs of *Ascaris*, resulting, in both cases, in the failure of normal partition walls to form.

9. Polyploidy is more frequent in plants than in animals, partly because of the fact that the polar centers are not definitely localized in the former.

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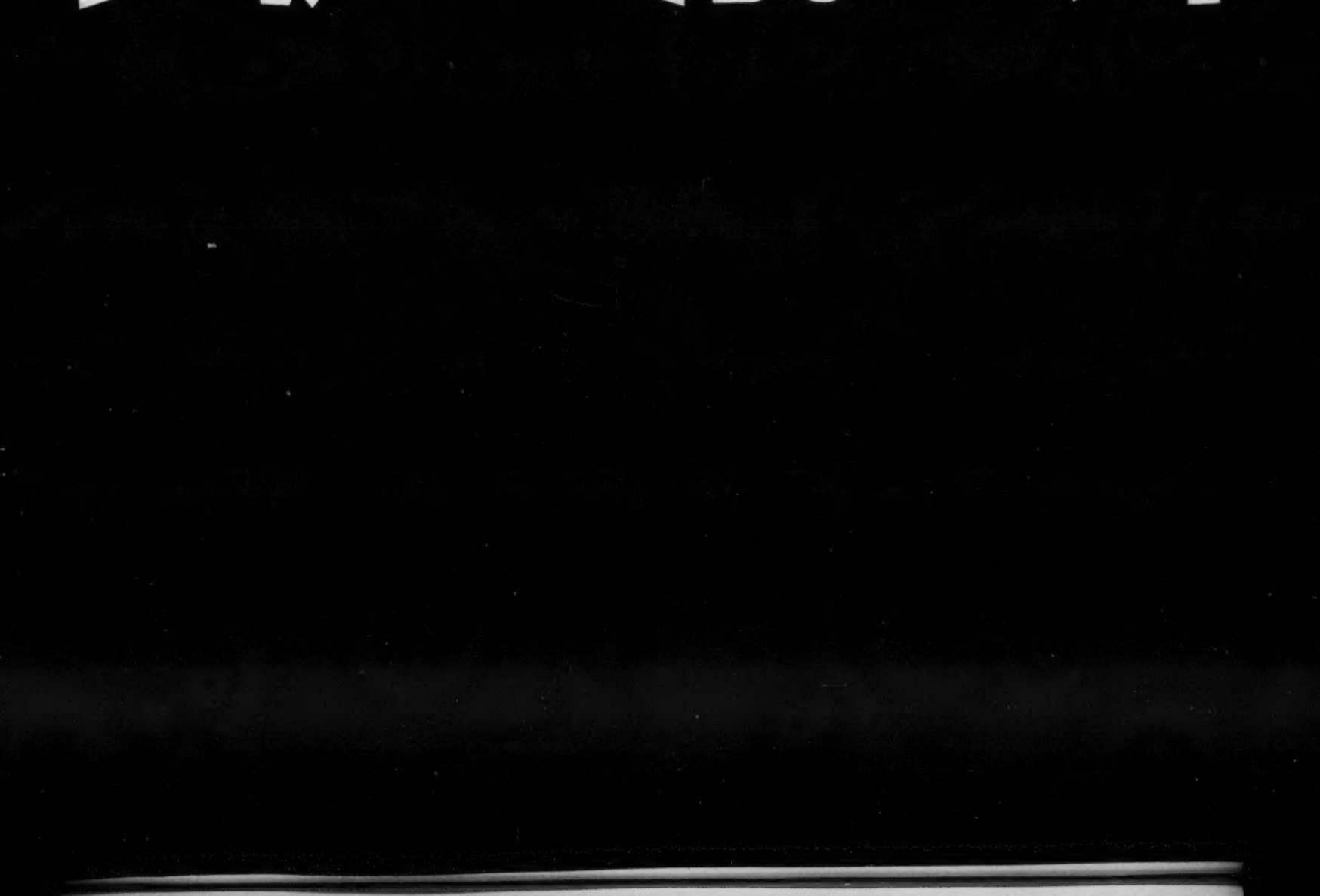
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